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OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

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NUMBER I

STUDIES ON THE ISLANDS OF LANGERHANS IN HUMAN PANCREAS *

I. THE RELATION OF THE ISLANDS TO THE SURROUNDING STRUCTURES

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INTRODUCTION AND HISTORICAL REVIEW

The question as to the morphologic relation of the islands of Langerhans to the surrounding pancreatic structures in the human and animal pancreas is still under discussion in the literature. One group of investigators (Helly,⁷ Weichselbaum and Kyrle,²³ Opie,¹⁷ Flint⁴ and others) claims that the islands generally have a limiting fibrous capsule and are, therefore, anatomically independent from the rest of the pancreatic tissue. The other group (Gellè,⁶ v. Hanse-
mann,⁸ Vincent and Thompson,²¹ Koch,¹³ Herxheimer,¹⁰ Fahr⁵ and others) however, maintains that the islands are throughout directly connected with the surrounding acini of the pancreas. Whereas Weichselbaum and Kyrle²³ and Helly⁷ concede that there are a few islands which are continuous with the ducts, Laguesse¹⁴ and Bensley¹ describe a constant continuity with the ducts as well as with the acini.

In the most recent literature Nakamura¹⁶ claims that the islands do not show real connection with the acini. Oertel¹⁸ and Vincent,²² however, even express the opinion that there is no morphologic proof at all to separate the islands, as organs with internal secretion, from the rest of the pancreas.

* Received for publication September 13, 1926.

It seems surprising to find such wide divergencies of opinion upon a simple morphologic question. If we inquire into the possible causes of the disagreement among the notable authors, we think that two factors may account for it, *viz.*, inadequate human material and insufficient technic.

In regard to the first factor, it is well known that the pancreas very rapidly undergoes severe postmortem changes, which fact seriously interferes with exact histologic studies. However, it is most difficult to secure human pancreas promptly after death because of institutional regulations. The second factor seems of far greater importance and must be discussed at some length. It is recognized that the pancreas readily shrinks and becomes brittle after routine paraffin preparation and that thin sections are not so easily obtained. The difficulty becomes serious if one intends to prepare serial sections, which is imperative in a question of morphologic relations between various structures. That such difficulties did actually interfere may be inferred from the fact that some of the authors do not illustrate their observations or do so only by inadequate drawings. It seems, however, that if photomicrographs are not available, camera lucida drawings should be presented to support the morphologic conclusions if they are to receive serious consideration.

Of the greatest importance in our opinion is the choice of the proper staining method, a point which has been neglected, with few exceptions. The question of the relationship between islands of Langerhans and surrounding structures centers in the question of the presence or absence of limiting fibers. Therefore, the solution of the problem rests upon the most exact method for their demonstration and on the examination of sufficiently long series of sections. Only the silver impregnation is capable of bringing out the whole of the finest tissue fibers. The claim can be made that our question can only be settled by examination of a considerable number of specimens in thin serial sections prepared with silver impregnation. Since this requirement has not been complied with, we decided to study anew the question of the relation between islands of Langerhans and surrounding pancreatic structures.

MATERIAL AND METHODS

The pancreases of forty-six individuals ranging in age from 3 days to 76 years were examined; 22 adults from 20 to 76 years, 13 children from 1 to 13 years and 11 babies from 3 days to 11 months. In all cases the tissue was removed from the body within five hours after death. In one-third of the cases sections from head, body and tail of the pancreas were examined, in the other two-thirds only one region was cut.

The tissue was fixed in 20 per cent formalin (8 per cent formaldehyde), Helly-Maximow and Lane's solution. The blocks were embedded in paraffin and cut in serial sections 3 to 5 microns in thickness, series averaging 30 to 40 sections. The series were stained by the Bielschowsky-Maresch silver impregnation slightly modified by the author. The impregnation was followed by hematoxylin-eosin stain. For comparison and detailed studies of the cellular granulations, hematoxylin-eosin, Bensley's neutral gentian and the azure B stain developed by MacNeal and the author were employed.

Silver impregnation method on paraffin sections, modified by the author. Fix pieces 3 mm. thick in 20 per cent formalin two to three days; wash thoroughly in running water over night; embed in paraffin.

1. Place the deparaffinized slide in 2 per cent silver nitrate solution in dark staining jar at 37 C for 24 hours or more until section is pale yellow.
2. Wash in distilled water one second.
3. Transfer the slide immediately into the ammoniated basic silver solution in dark staining jar and leave it for one hour at room temperature.

This solution must be freshly prepared each time, as follows: To 20 cc. of 1 per cent silver nitrate solution add 1 drop of 40 per cent sodium hydroxide. Let the test tube stand one minute without shaking and then add 2 to 3 drops of strong ammonia (28 per cent). Shake the test tube slowly. If the solution is not clear, add one more drop of ammonia. The precipitate should not be dissolved entirely; however, the supernatant solution must remain clear. Heat the solution to about 50 C. Filter into dark staining jar. The slide must be placed in this solution while it is still warm. (To obtain a constant satisfactory result, the ammonia solution should be kept in a bottle with a paraffined glass stopper.)

4. Wash in distilled water one second and immediately put the slide into 2 per cent formalin, 1 to 3 minutes.
5. Wash in water and stain with hematoxylin-eosin.

Note: As the precipitate after impregnation usually does not occur in the sections, it is not necessary to use gold chloride solution.

The description of the histologic findings was made from the silver preparation.

HISTOLOGIC OBSERVATIONS

Before entering upon the questionable relation between islands and surrounding pancreatic structures we have to make mention of the arrangement of the fibrillar framework within the pancreas, as we have observed it with the silver impregnation.

Generally the acinus is surrounded by fine black fibers which form a latticed network, as can be recognized in serial sections (Fig. 9). These fibers serve as the basement membrane of the acinar epithelium and as the supporting framework of the blood capillaries. Between acinus and island, fibers of the same caliber are seen (Figs. 3 and 15); therefore they can be recognized as the basement membrane of acini. Branches of these fibers, however, extend into the island and circumvene the insular capillaries. Similar fibers can be found around the excretory ducts.

According to our observations the islands in the specimens examined can be divided into three types: A, islands continuous with acini; B, islands directly continuous with ducts; and C, islands strictly separated from the surrounding tissue.

Type A. Islands connected with acini without intervening fibers. This type was found in every specimen of our series though in varying frequency. A smaller or larger segment of the insular circumference may be continuous with the surrounding acinar cell groups. The following cases may illustrate that type.

Case 17.* Male. 52 years old. Diagnosis: Bronchopneumonia; arteriosclerosis; chronic endocarditis.

A medium-sized island was followed in nineteen serial sections 5 microns thick. In three sections the island is sharply separated from the surrounding acini by their limiting basement membranes. In the other sixteen sections, however, the insular cells are in direct contact with surrounding acini.

Fig. 1A shows at the left side of the island an acinus separated from the insular cells by fine fibers. In the next section, however, Fig. 1B, the limiting fibers are interrupted and the acinar cells blend with the insular cells. Similar conditions are seen on the opposite (right) side of the island. The pictures present the one extreme in which the island is almost completely separated from the surrounding acini. Only serial sections and the demonstration of the small

* Case number refers to our protocol.

breaks within the limiting fibers permit a correct interpretation. The majority of the islands in this case show a similar structure but with considerable variation in the extent of the continuity between insular and acinar cell groups. However, one finds occasionally a small island which is completely separated from the surrounding structures without any break in the limiting fibers in same section. (See left side of Fig. 1A and 1B.)

Case 13. Female, 9 years. Diagnosis: Tumor cerebri; lobar pneumonia.

Here islands are found which showed no limiting fibers at all around the insular circumference, the insular cells freely blending with the acinar cell groups (Fig. 2). This picture represents the other extreme of Type A.

Case 82. Male, 27 years. Diagnosis: Postoperative shock (tenorrhaphy of left hallux).

Fig. 3 shows the one extreme in which the island is connected with acinar cell groups in moderately wide segments; these pictures have been found most frequently.

Case 55. Male, 3 months old. Diagnosis: Adrenal tumor.

Fig. 4 shows an identical picture.

Islands of Type A within the limits of the extremes represented in Figs. 1, 2 and 3 were found in great numbers in all cases examined. In fact it is the type most frequently observed in our material.

Type B. Islands connected with ducts. Here we may distinguish two sub-groups: (a) The area of continuity between islands and ducts is very narrow; (b) the duct and island are in broad communication.

Case 13. Female, 9 years. Diagnosis: Tumor cerebri; lobar pneumonia.

At the periphery of a lobule a medium-sized, oval island is found in connection with a duct. Followed in serial sections one can see that the epithelial lining of the duct passes into the insular cell groups at one end. There are no limiting fibers found throughout the series (Fig. 5). Other similar islands are found at the periphery of the lobules. There is no evidence of pathology of the pancreas.

Case 72. Male, 3 years. Diagnosis: Pneumonia; otitis media.

A very small island is connected with the epithelium of a dilated duct (Fig. 6).

Case 67. Male, 11 months. Diagnosis: Bronchopneumonia.

Fig. 7 shows a branch of an interlobular duct leading directly into an island. Note the absence of limiting fibers at the area of contact.

Similar conditions have been frequently observed in the pancreas of infants.

Case 73. Male, 71 years. Diagnosis: Hemorrhagia cerebri; cirrhosis of the liver; arteriosclerosis.

Fig. 8 shows a duct with two islands attached. Whereas one side shows intact epithelial lining separated from the island by several layers of fibers, the opposite side shows a wide break in the limiting fibers and communication with the insular cells. The next section shows limiting fibers on both sides.

Similar observations were made in three further cases.

Type C. Islands entirely separated from the surrounding pancreatic structure.

Case 64. Female, 32 years. Diagnosis: Postoperative peritonitis.

In this case a considerable number of islands appear completely separated from the surrounding acini by fine fibers. One of these islands was studied in twenty-two serial sections, 4 microns thick. It is round and in the periphery of a lobule attached to an interlobular excretory duct. No break in the limiting fibers is observed as evidenced in the picture of the series (Fig. 9). In this case there is proliferation of the fat tissue surrounding the pancreas which even grows between the lobules (lipomatosis). There is, however, no evidence of previous inflammation, although the fibers are slightly thicker than usual, most probably due to the slight atrophy of the acini. In this case three medium-sized and three very small islands with identical structure are found; the majority of the islands, however, show continuity with the acini, though to a rather small extent of the circumference (Type A).

Case 8. Male, 34 years. Diagnosis: Peritonitis after perforated ulcer of duodenum.

Two adjoining islands within a lobule are entirely separated from the surrounding acini by fine fibers. No inflammatory changes or increased connective tissue are found within the pancreas (Fig. 10).

Case 34. Male, 1 year. Diagnosis: Bronchopneumonia.

Similar islands are found and one followed in twenty-two serial sections, 4 microns thick. The island is completely separated from the surrounding structures (Fig. 11). No evidence of pathologic changes in the pancreas.

Case 80. Male, 59 years. Diagnosis: Pneumonia.

The pancreas shows marked lipomatosis. Within the fat tissue a great number of isolated islands are found. In serial sections they prove to be entirely separated from the surrounding tissue (Fig. 12).

Case 28. Female, 46 years. Diagnosis: Embolism of pulmonary artery; status post-suspensionem uteri.

Numerous islands are entirely separated by very thick connective tissue fibers from the surrounding acini (Fig. 8). The marked general fibrosis of the septa between the lobules is conclusive evidence of previous inflammation (Fig. 13).

This type of island is found only after considerable search.

Among these three types of islands, Type A is the most frequent, while the others are found only after considerable search. The three different types occasionally occur in one and the same pancreas in fairly identical proportions, even in one section. Generally, however, one type predominates. This fact is clearly demonstrated in Figs. 14 and 15. The former shows large, irregularly outlined islands directly connected with acini. The latter, however, contains round or oval islands, for the most part strictly separated from the acini. Occasional islands show direct contact with the acini but only in a small segment of the circumference.

Islands of Type B are connected with interlobular or intralobular pancreatic ducts. However, it seems self-evident that the islands connected with acini are *eo ipso* continuous with the terminal ducts. That feature, however, is not clearly recognizable because the terminal ducts are mostly collapsed.

In regard to size and shape we find great variations among the islands. The distribution varies considerably in different cases. In four cases we found groups of numerous islands within the head of the pancreas, while the other portions showed a fairly even distribution.

*

COMMENT

According to the relation of islands to the surrounding pancreatic structures we have found the usual occurrence of three types in the human pancreas. Our observations differ from the results of nearly all former investigators who described the occurrence of only one type of island. Our studies corroborate Laguesse,¹⁴ however, in his opinion that the islands are continuous with the acini as well as the

ducts and prove the occasional occurrence of strictly separated islands, a fact which was only surmised by him.

We attribute the difference in our findings to the method employed and the number of cases examined. We have based our conclusions exclusively on the actual findings in the postfetal pancreas.

Our observations have conclusively shown that the type of islands which are in direct contact with the surrounding acini is the most frequent one found in our material. That such a frequency is not the result of pathologic changes in the pancreas is evidenced by the fact that our observations include a great number of newborn and young children and that identical conditions were found by us in healthy animals such as rabbits, guinea-pigs, hens and pigeons. We may even add that children show a distinct prevalence of this type. This fact also argues against the contention of Weichselbaum and Kyrle,²³ Helly,⁷ Opie,¹⁷ Nakamura¹⁶ and others, who refuse to accept the occurrence of connections between islands and acini, because such connections occur only in a very early period of fetal life and disappear later.

Notwithstanding the fact that the histologic methods employed by the above-mentioned authors are open to argument, the predominance of islands of Type A in the pancreas of newborn babies and children indicates that the structure of the pancreas undergoes further changes in the early postfetal period.

Islands connected with ducts range second in our material in regard to their frequency. Weichselbaum and Kyrle and Helly have described the rare occurrence of that insular type in normal pancreas, whereas Opie,¹⁷ Pearce,¹⁹ Karakascheff,¹¹ Seyfarth,²⁰ Nakamura¹⁶ and others have demonstrated connection between islands and ducts in cases of congenital syphilis. Our observations are more in accord with those of Laguesse¹⁴ who maintained that such an occurrence is rather the rule than an exception. Similar opinion is held by Bensley¹ for the pancreas of the guinea-pig.

The existence of islands entirely separated from the surrounding pancreatic tissue could be demonstrated beyond doubt in five specimens of normal pancreas. Whereas Weichselbaum and Kyrle and Helly and others maintain that this type of island is commonly found, Herxheimer¹⁰ and Seyfarth²⁰ believe that it only occurs in pathologic organs, as in fibrosis. Our observations, while contradicting the latter view, fail to confirm the former statement, because

the separated type of island was the least frequent in our material although its occurrence cannot be denied. However, we have frequently seen islands apparently completely separated from the surrounding structures in which only the study of complete serial sections revealed a minute connection with an acinus. Such pictures suggest the possibility that islands of this type might easily become completely separated because there is no doubt that such completely separated islands are very frequent in acquired pathologic conditions of the pancreas, as fibrosis or lipomatosis.

The question as to the existence of a fibrous capsule of the island has been frequently discussed in the literature without definite agreement. Since we conceive the limiting fibers around the islands as the basement membrane of the acini it is self-evident that the occurrence of a thick collagenous fibrous capsule is conclusive proof of a pathologic condition of the pancreas.

SUMMARY AND CONCLUSIONS

1. The pancreases of forty-six individuals ranging in age from 3 days to 76 years were studied in serial sections stained with silver impregnation in order to determine the relation of the islands of Langerhans to the surrounding pancreatic structures.

2. Three types of islands were recognized in the normal pancreas: A, islands connected with surrounding acini, found in every case; B, islands connected with interlobular and intralobular ducts; and C, islands strictly separated from the surrounding structures. The classification of the types was made in the order of their frequency.

3. The islands have no fibrous capsule of their own but a more or less complete separation is produced by the basement membrane of the acini or ducts or by the interlobular connective tissue.

4. There are wide variations in size, shape and relation of the islands to the surrounding pancreatic structures. Such differences are present not only in the same organ but even in the same sections. Therefore, it is improper to speak of one regular type of the island of Langerhans in respect to this relation.

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DESCRIPTION OF PLATES

All preparations illustrated except Figs. 12 and 13 were made by the silver impregnation method.

PLATE I

- FIG. 1. An oval island shows direct connection with acinar cell groups in a small segment of the circumference. See the description on p. 4. A small round island which has been followed in 14 serial sections is entirely separated from the acini.
- FIG. 2. An island connected with the acinar cell groups directly in the greater part of its circumference. There are no limiting fibers at all around the island.

PLATE 2

- FIG. 3. An island connected with the acini in a moderate portion of its circumference.
- FIG. 4. Islands not limited from the acini.

PLATE 3

- FIG. 5. An island connected with a duct.
- FIG. 6. An island connected with a duct.

PLATE 4

- FIG. 7. Undifferentiated insular cell groups connected with a branch of a duct.
- FIG. 8. An island connected with the epithelial lining of a duct.

PLATE 5

- FIG. 9. An island everywhere strictly separated from the rest of the pancreatic tissue by fine fibers. A complete series.

PLATE 6

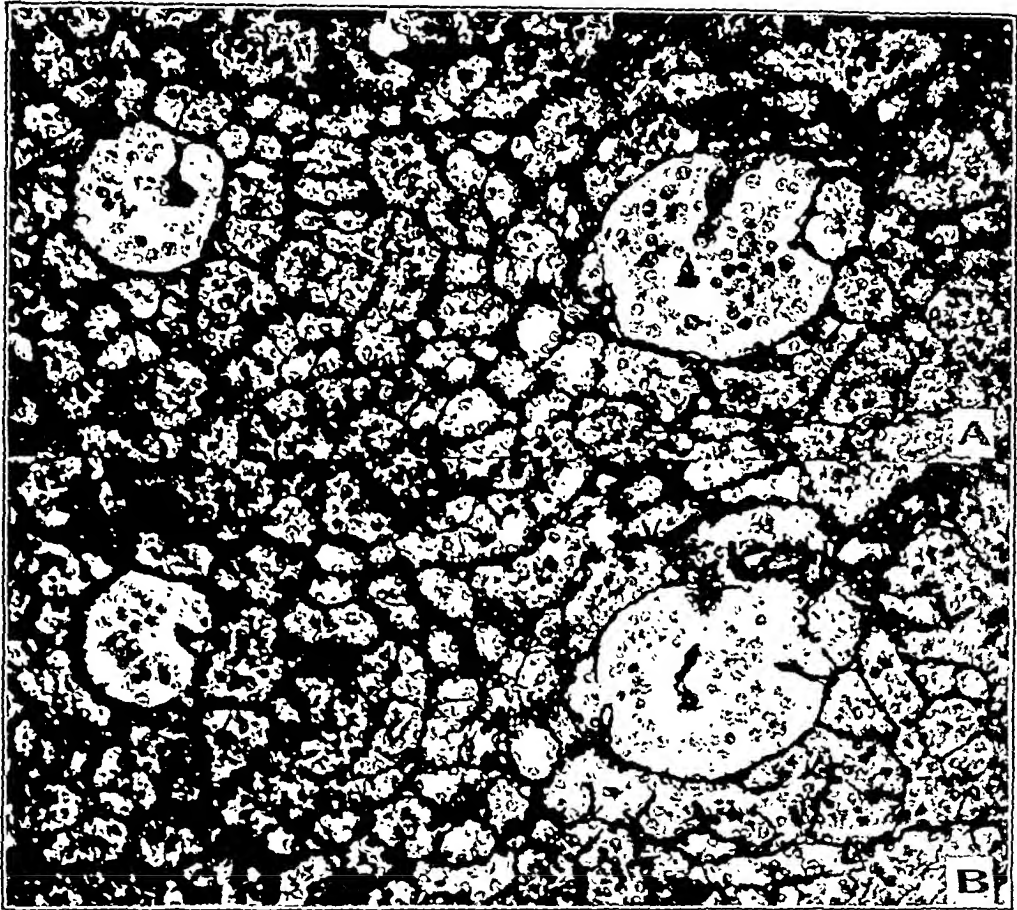
- FIG. 10. Islands sharply separated by fine fibers.
- FIG. 11. One island (in the center) is sharply separated; the other islands show direct connection with acini.

PLATE 7

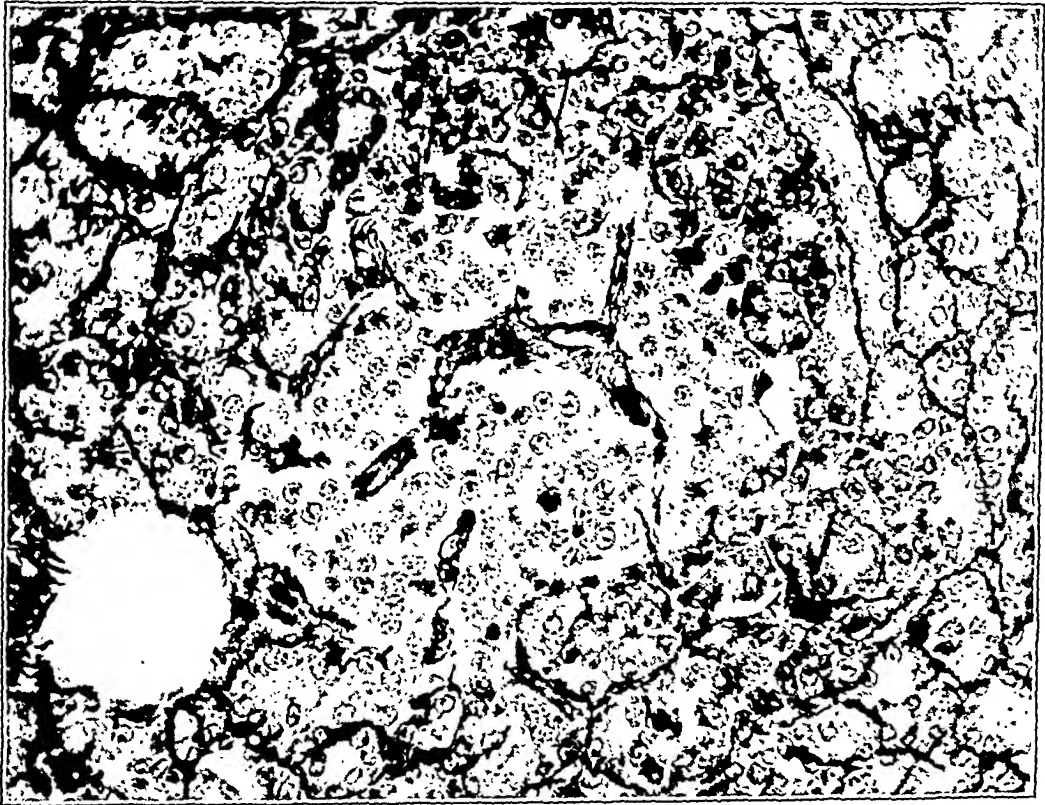
- FIG. 12. The islands which remain completely separated in the fat tissue (lipomatosis) (followed in serial sections). Hematoxylin and eosin.
- FIG. 13. Islands surrounded by thick connective tissue fibers. Hematoxylin and eosin.

PLATE 8

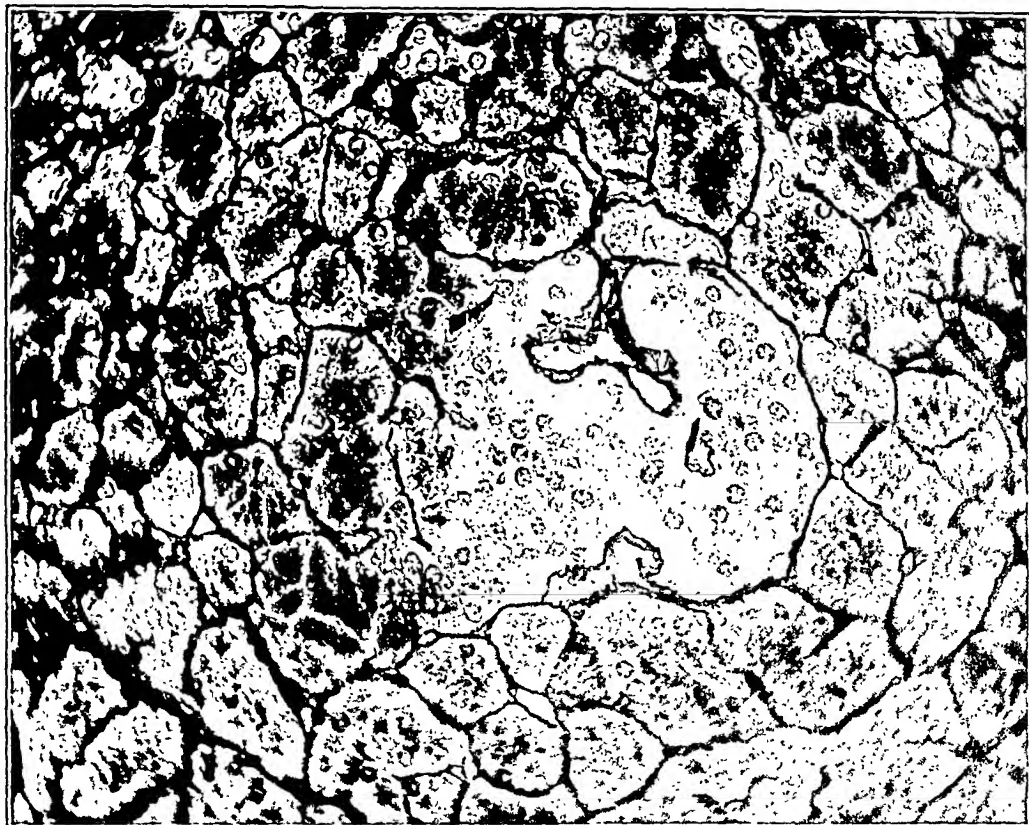
- FIG. 14. Pancreas with irregular form of the islands connected with acini.
- FIG. 15. Pancreas with rather round islands, most of them separated from the rest of the pancreatic tissue, while few islands are connected with the acini.



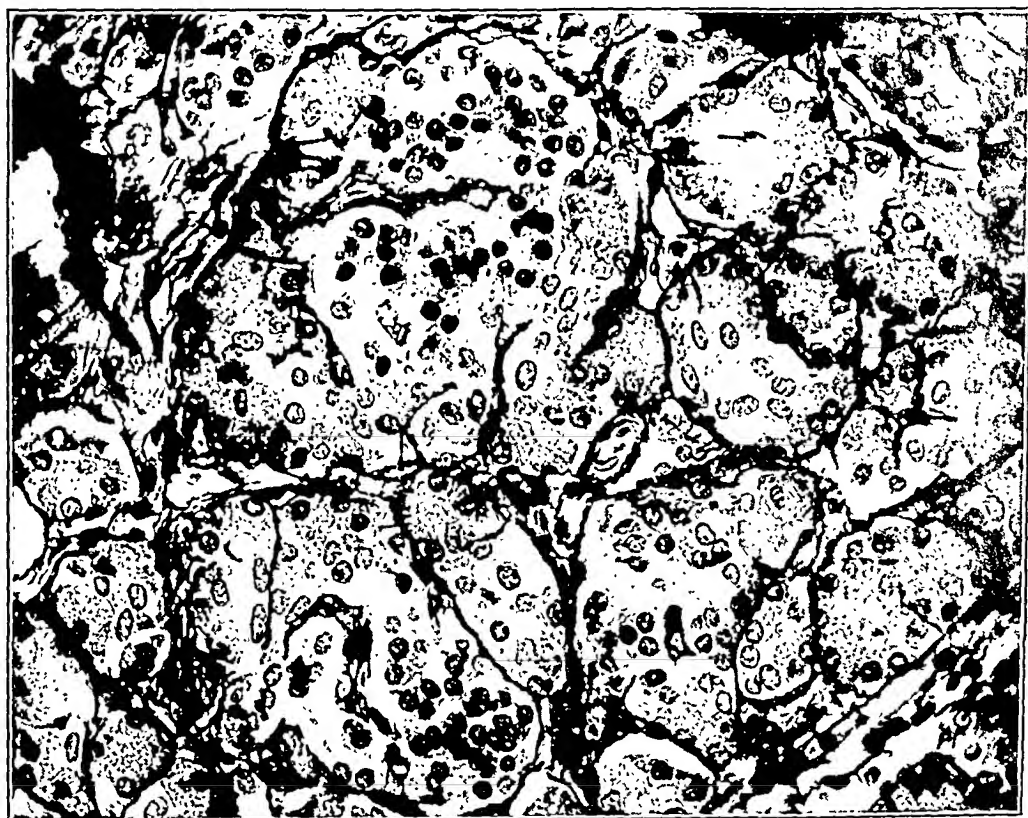
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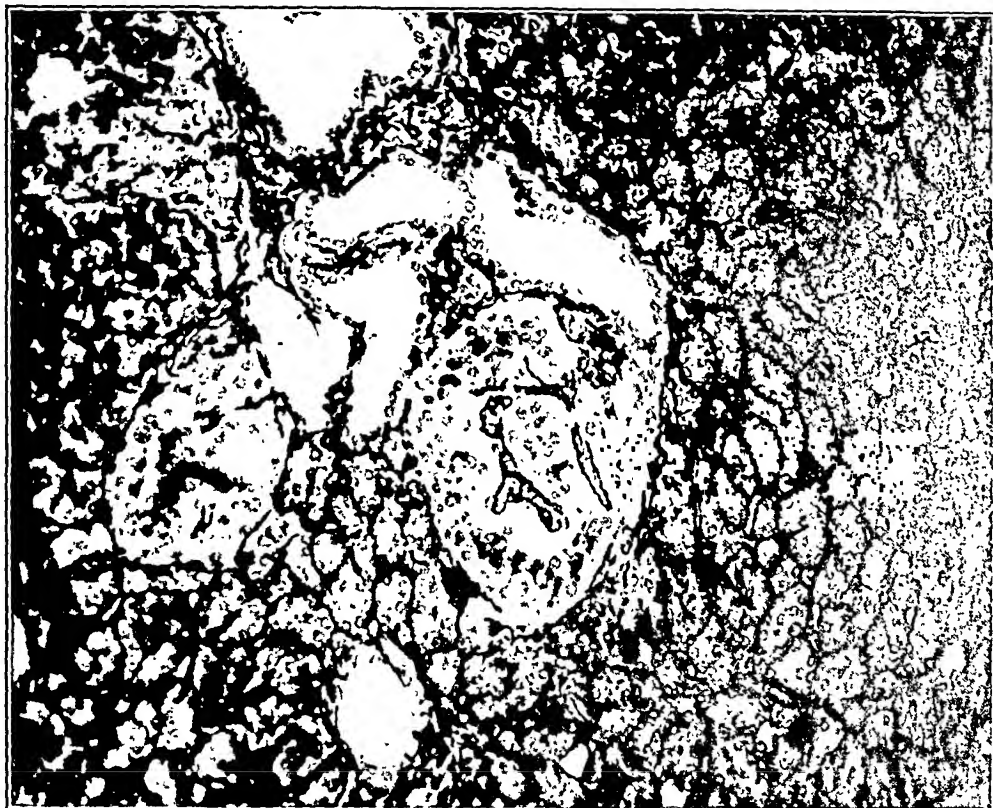
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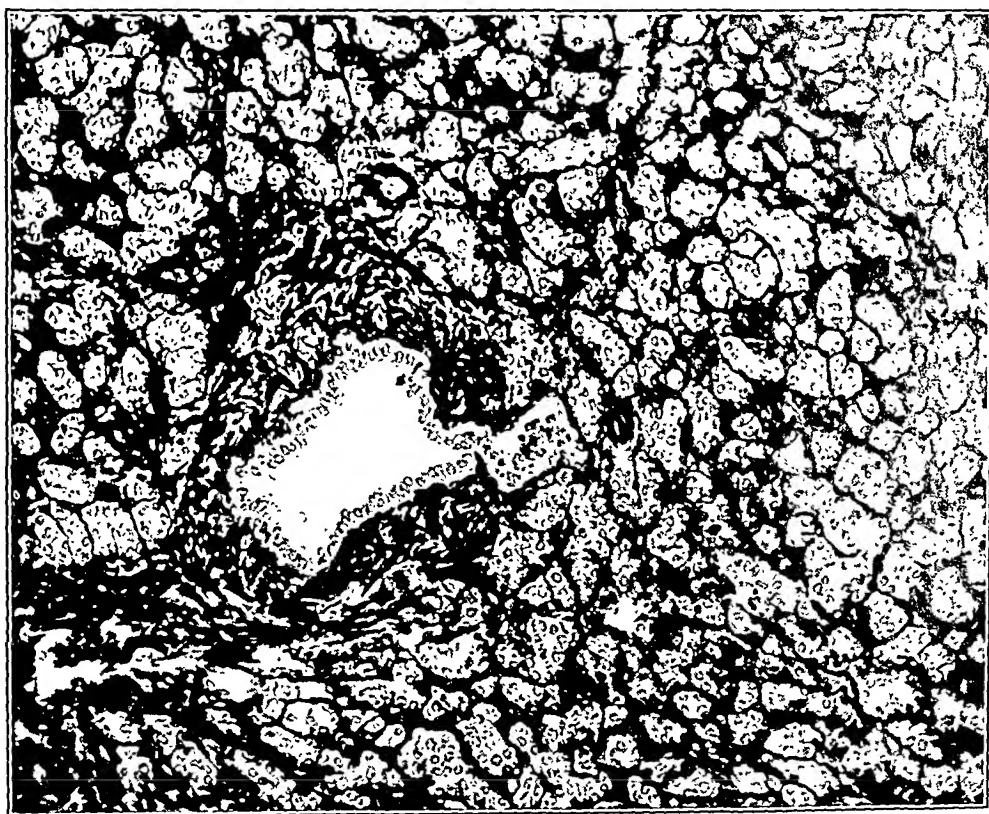
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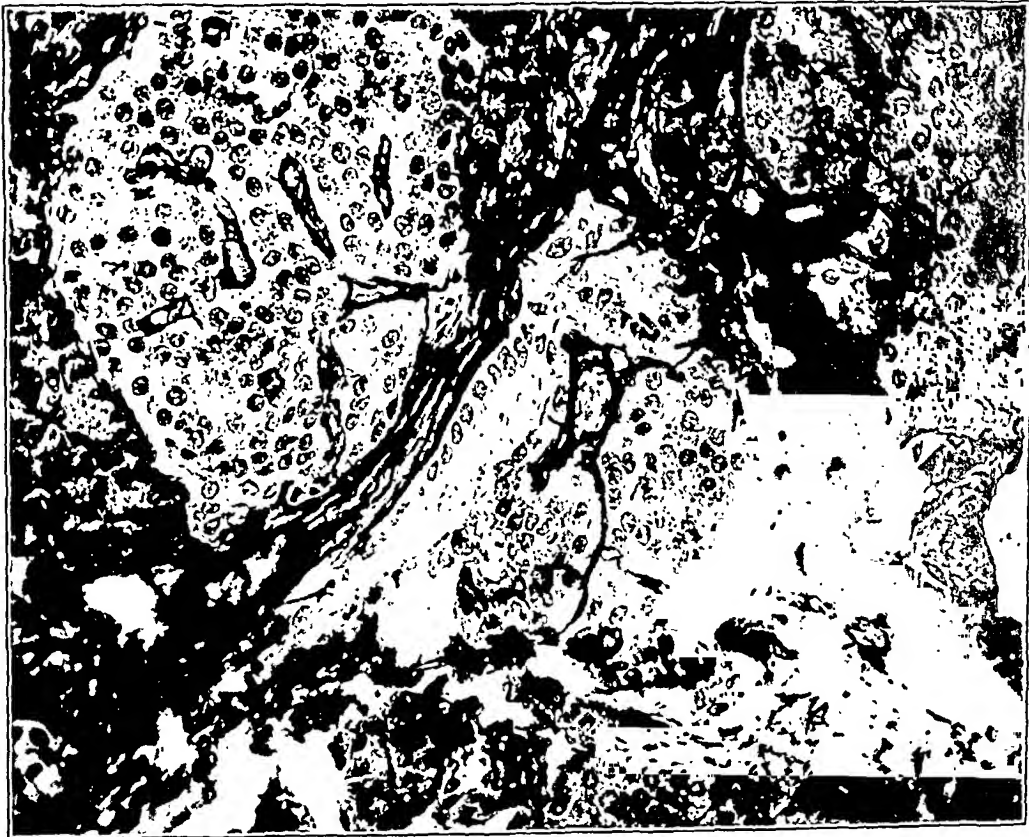
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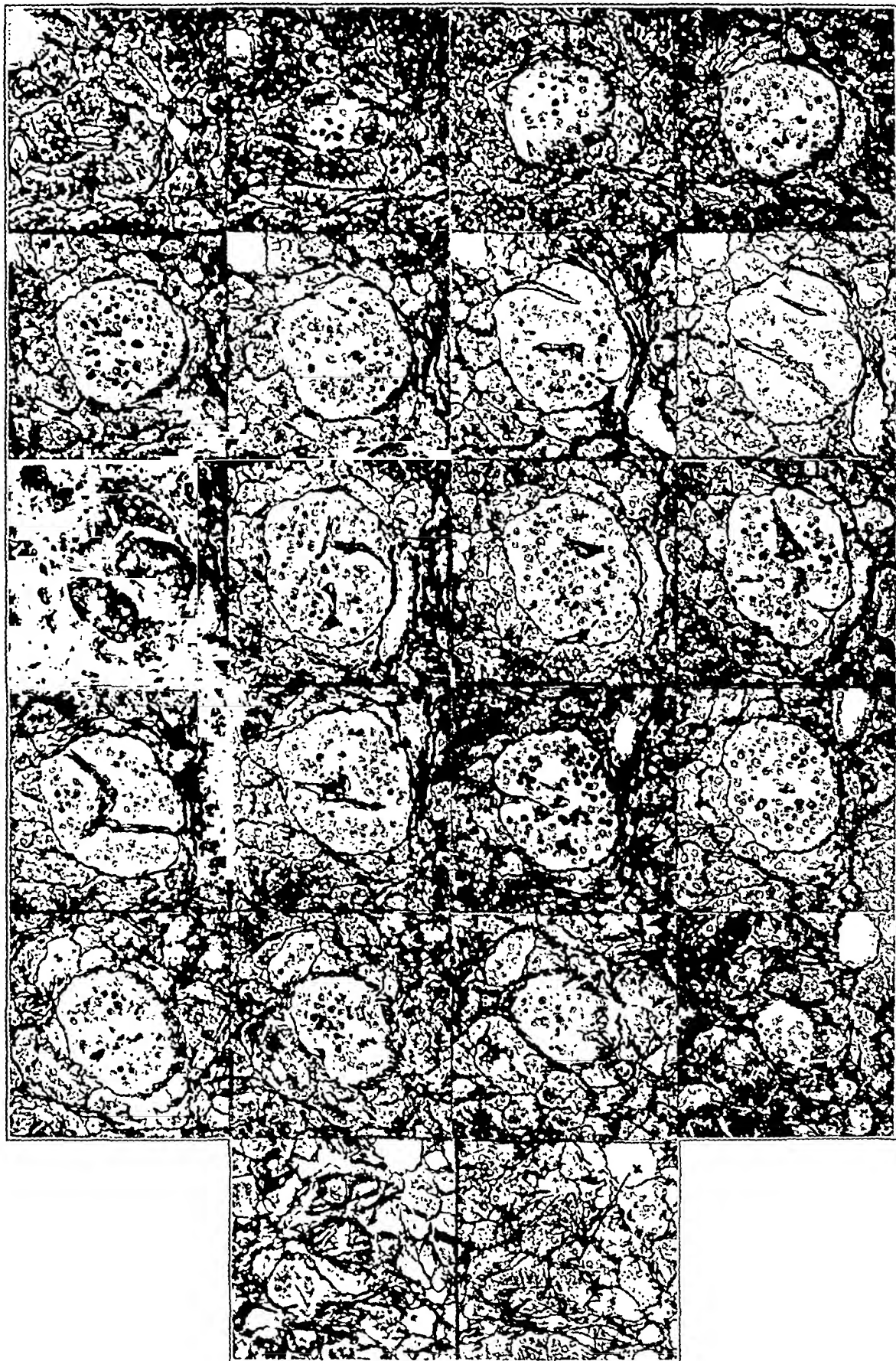
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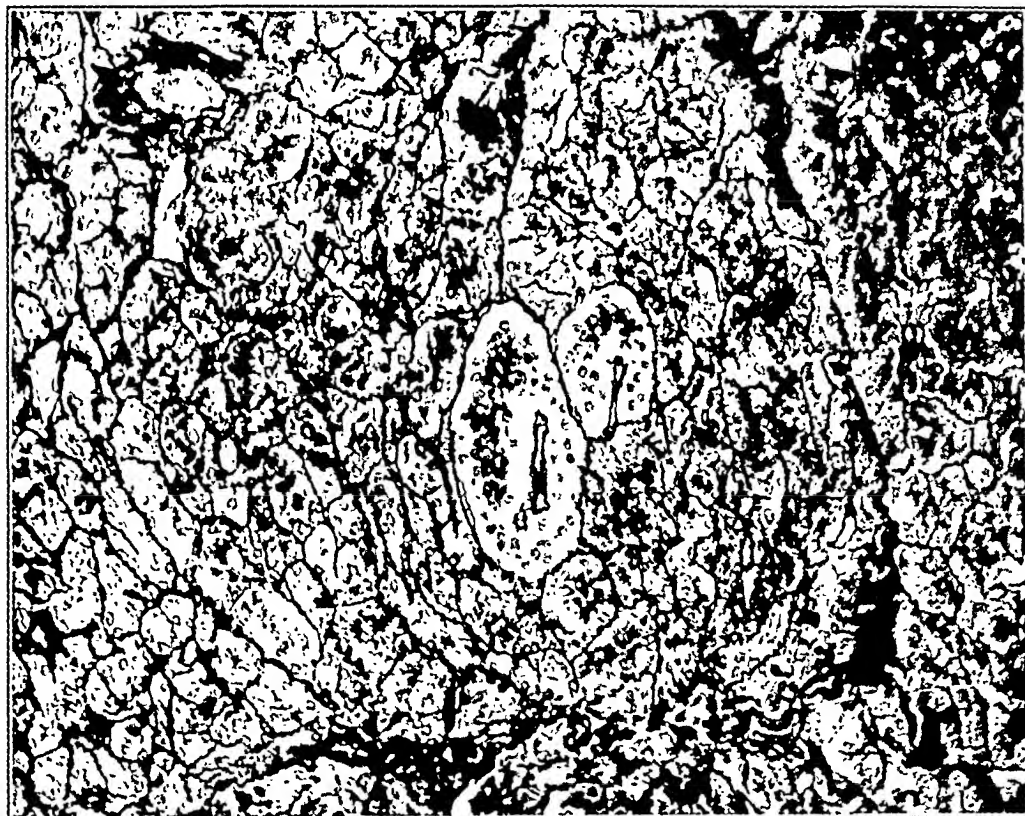


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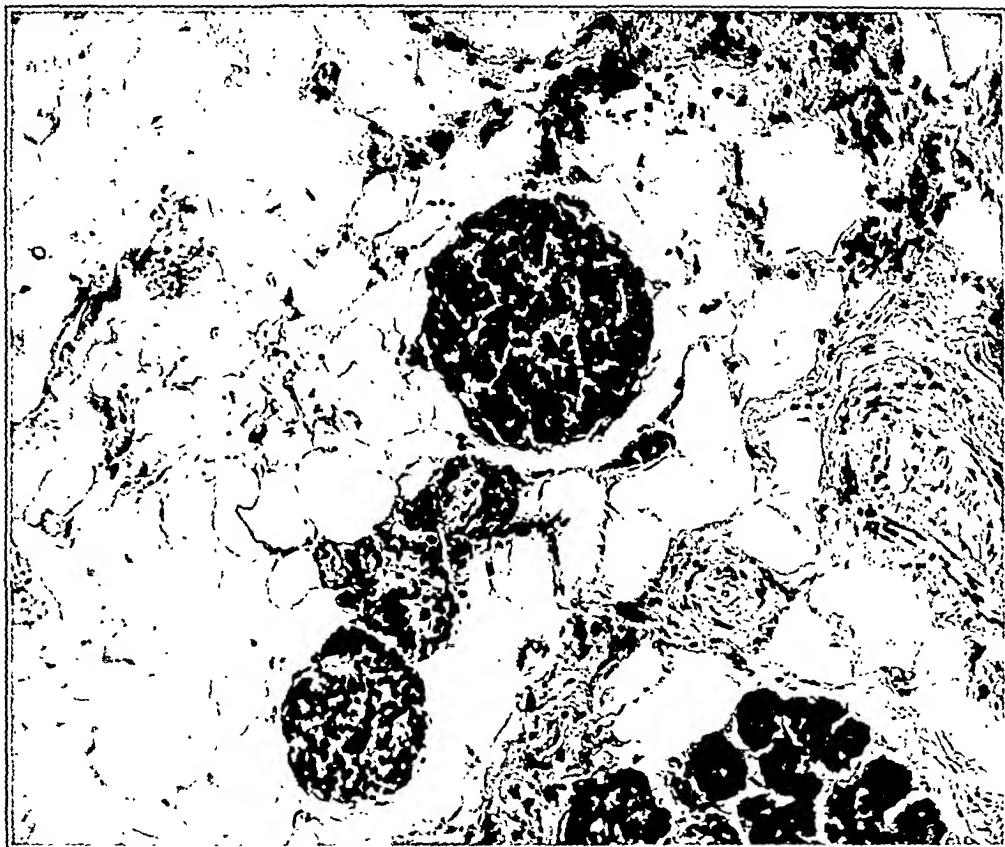




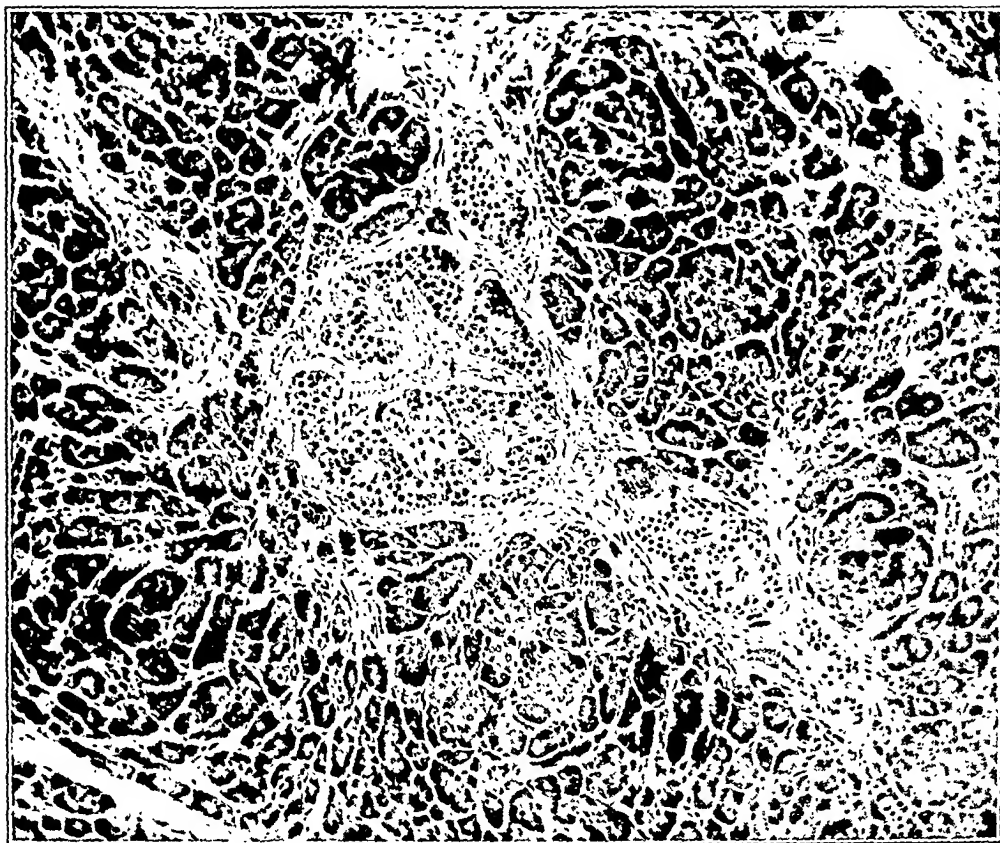
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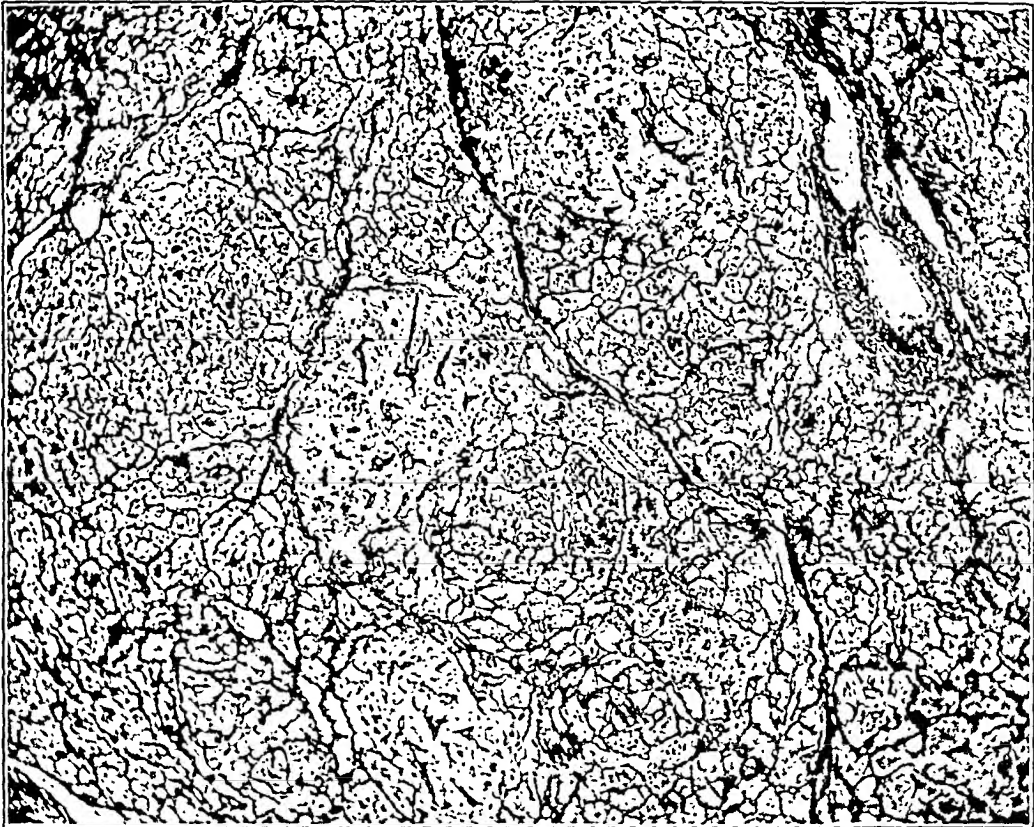
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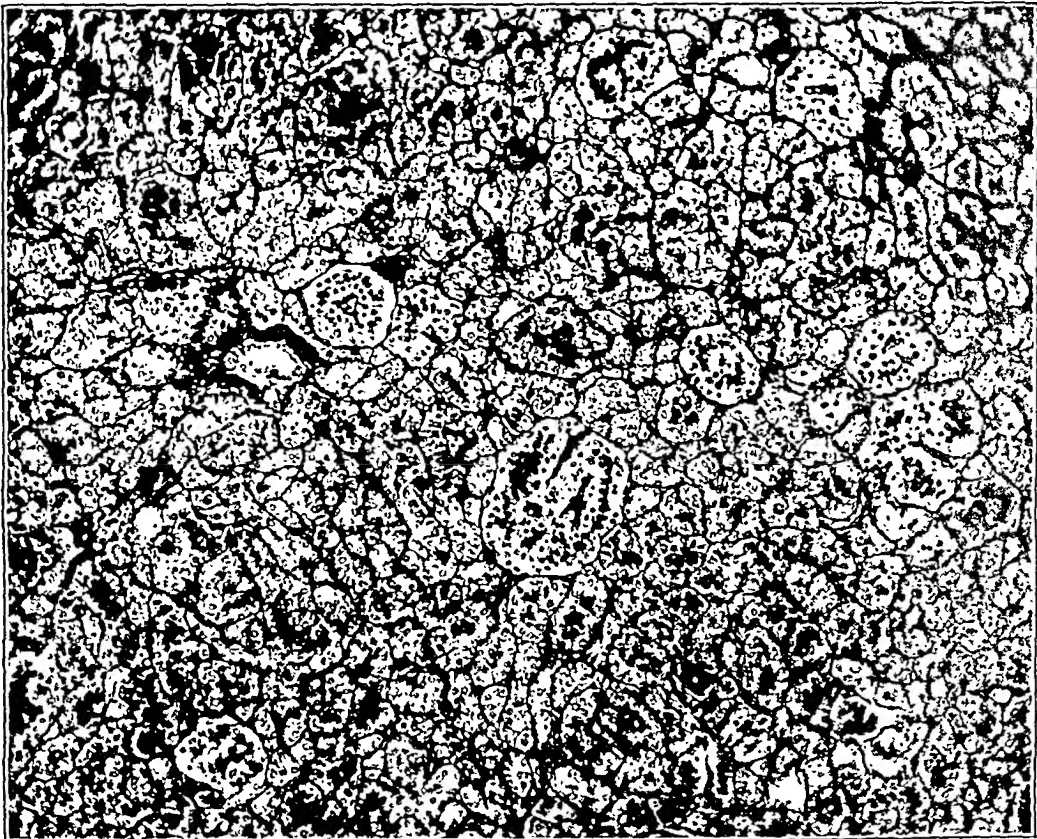
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14



15

THE ORGANIZATION OF EXPERIMENTAL ADRENAL CELL EMBOLI IN THE LUNGS *

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In the course of experiments intended to produce a cytolytic serum for adrenal cells, a somewhat coarse emulsion of dog adrenal tissue was injected into the ear vein in a number of rabbits. As was expected small emboli composed of groups of adrenal cells lodged in the lungs. These emboli did not of themselves cause death of the animals, although some of them died very quickly after the third or fourth intravenous injection just as they sometimes do in any attempt to produce antibodies to other antigens by this method. From these animals a series of lungs was obtained containing emboli from a few minutes to two weeks old.

The organization of these adrenal cell emboli was characterized by a reaction so different from that of ordinary emboli that it has seemed worth while to present this brief report.

In the earlier stages numerous small blood vessels in the lungs were found plugged with finely granular material with a few pyknotic nuclei and vague adrenal cell outlines. In some cases the adrenals had been preserved in glycerine for several days before being ground up or injected into the rabbits. This accounts for the partially necrotic condition of the adrenal cells found in the pulmonary vessels of those animals that died immediately after the intravenous injection.

On casual low-power examination, those emboli in which the process of organization was well advanced resembled tubercles. The older ones contained one or more giant cells with four to twenty or more nuclei irregularly scattered through the cytoplasm, thus resembling foreign body giant cells rather than typical Langhans cells. The central part of the organizing mass was frequently necrotic but this central area was composed of dead adrenal cells and not of necrotic, newly formed tissue as in a tubercle. The greater number

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of elements in this mass, however, were large, oval or spindle-shaped cells with abundant cytoplasm, rather indefinite outlines and round, oval or indented vesicular nuclei, thus resembling the epithelioid cells of a tubercle. Surrounding this pale central mass of epithelioid and giant cells was a zone of densely crowded lymphocytes. In several of the animals eosinophiles were present in great numbers in the outer edges of the lesions and the immediately adjacent lung tissue. In some instances the organizing mass appeared to have become canalized for it showed a small blood-filled channel usually eccentrically located.

Much the same type of change occurred in the process of organization of a similar emulsion of adrenal tissue injected subcutaneously into rabbits. In the latter location, however, an Arthus phenomenon of an especially violent type was produced after two to four subcutaneous injections of emulsion of dog adrenals at intervals of two to three days. This occurred much more constantly and more promptly than after the injection of horse serum. In the subcutaneous tissues, therefore, the process of organization of the adrenal fragments was complicated by the process of necrosis and inflammation incident to Arthus phenomenon. However, even here the same general type of reaction (formation of giant cells, epithelioid cells and accumulation of lymphocytes and sometimes of eosinophiles) could be readily distinguished.

In the organization of thrombi, of emboli originating from thrombi, of infarcts and of transplants of various tissues other than adrenal, epithelioid and giant cells may or may not take part in the process. They are specifically mentioned by Loeb¹ in the organization of transplants of kidney, liver and spleen. They are not referred to by Fleisher² and in other papers by Loeb in association with kidney transplants. Loeb does not mention them in relation to transplants of thyroid,³ skin¹ and corpus luteum.¹ He observed them in the organization of blood clots, being most numerous at places where the clot was especially dense and offered resistance to the progress of the invading fibroblasts. Karsner and Dwyer⁴ described them in organizing infarcts of the myocardium.

While epithelioid and giant cells are often present in the processes of organization just mentioned, they do not play such a predominant rôle as in the replacement of the emboli of adrenal cells described above. They have been observed by others in transplants of adrenal

tissue. Poll ⁵ saw large cells with as many as ten nuclei in adrenal transplants. He thought that they probably originated from adrenal cells because Manasse ⁶ had described multinuclear cells in "hyperplastic adrenal tumors." Schmieden ⁷ observed similar cells in adrenal tissue implanted in the kidney and believed that some of them were derived from the transplanted cells and that others were true foreign body giant cells. Stoerk and von Haberer ⁸ grafted bits of adrenal with vessel attached beneath the capsule of the kidney and found giant cells both in the renal tissue and in the transplant. They also mention cholesterol slits surrounded by foreign body giant cells.

The reaction, above described, of the lungs of these rabbits to small masses of adrenal cells lodged in their pulmonary vessels is distinctly of the foreign body type. This form of reaction is believed to be due to the chemical nature of the emboli. The formation of foreign body giant cells about crystals of cholesterol in the tissues is well known. Wells ⁹ found that 36.3 per cent of the dried adrenal gland consisted of ether-soluble material of which 20.6 per cent was cholesterol and 33 per cent phospholipins. Long ¹⁰ has stated that approximately 40 per cent of the dry weight of the tubercle bacillus consists of fat-like substances (lipins) but it apparently contains no cholesterol. There are, therefore, certain relationships between the chemical composition of the adrenal emboli and that of tubercle bacilli. This may be the basis for the similarities between tubercles and the nodules produced by the organization of the adrenal emboli in the lungs of these rabbits.

SUMMARY

Certain resemblances are pointed out between the histologic tubercle and nodules produced in the lungs of rabbits by the organization of experimental adrenal cell emboli. This peculiar type of reaction is believed to be due to the high content of lipid substances, especially cholesterol, contained in the adrenal cells.

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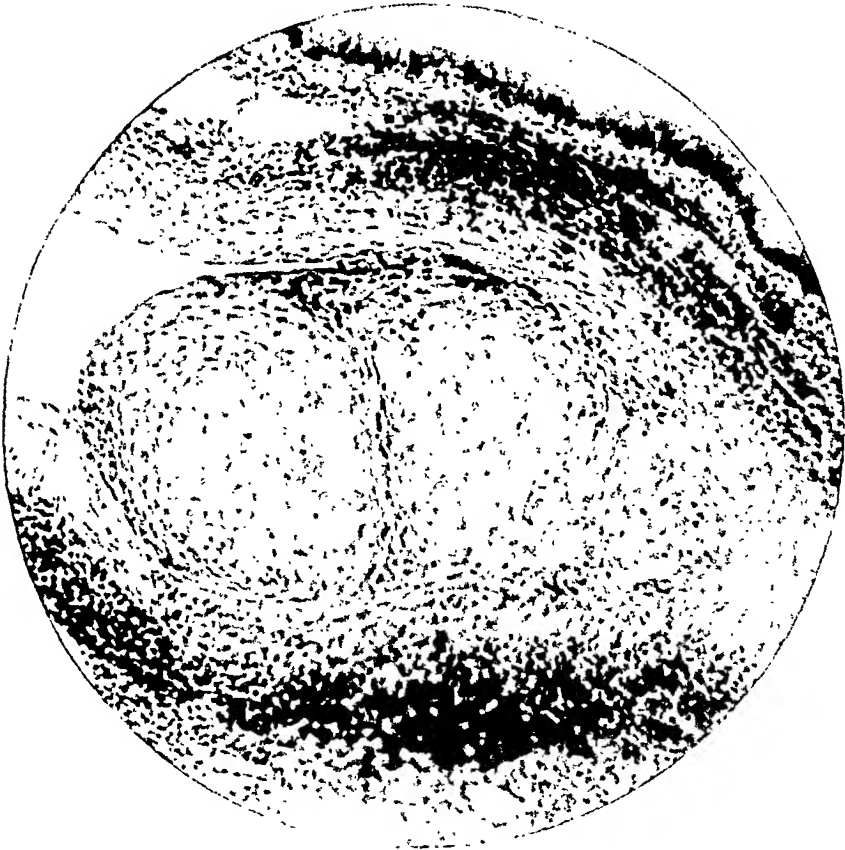
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DESCRIPTION OF PLATES

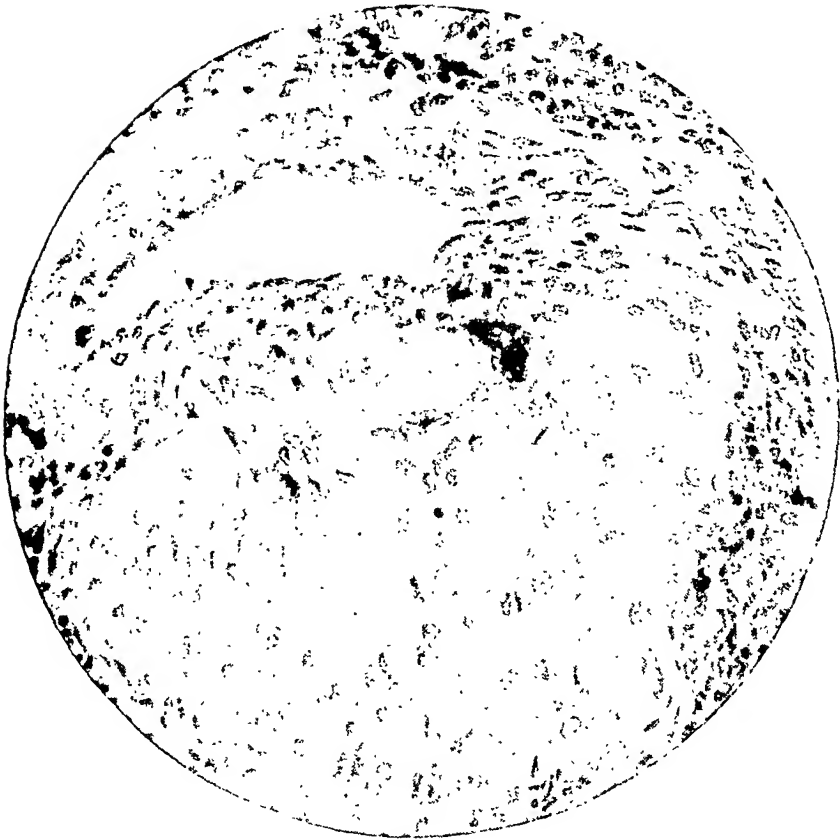
PLATE 9

FIG. 1. Organization of adrenal cell embolus. Diagonal section. $\times 170$.

FIG. 2. Organization of adrenal embolus. Transverse section. Cholesterol slits not in focus, to right of giant cell. Eosinophiles above giant cell. $\times 350$.



1



2

A QUANTITATIVE STUDY OF THE HYPOPHYSIS OF THE HUMAN ANENCEPHALIC FETUS *

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INTRODUCTION

The question of the presence or the absence of the hypophysis in the anencephalic fetus has been of special significance in connection with the subject of the function and interrelationship of the ductless glands. Some have even attributed this abnormality to a disturbance of the endocrine system. Previous to 1921 the general belief was that an hypophysis was frequently absent in anencephalics and when present was usually described as being different from the normal gland. No quantitative methods have been applied, consequently the literature is based on qualitative observations only.

It is the purpose of this investigation to apply quantitative methods to these abnormal specimens and when the organ is present to compare it with that of the normal. In order to bring the subject to its present status the principal contributions are summarized below.

REVIEW OF THE LITERATURE

Ballantyne¹ has described forty-five anencephalic fetuses and states that "the hypophysis rarely occurs." Schwalbe² says the hypophysis is sometimes present and in a large number of cases there is accessory hypophyseal tissue. Haberland³ examined three cases of anencephaly and reported an hypophysis as present in each. In one instance a pars nervosa was found in the upper part of the cranio-pharyngeal canal. The glandular portions, in this instance, extended through the canal and only a small polyp was present at either end. Brown⁴ examined five specimens of anencephalic fetuses and failed to find the hypophysis. He concluded, therefore, that the condition of apituitarism was normal for anencephaly.

Mauksch⁵ found an hypophysis in nine cases of this monstrosity. In five of these, he described a pharyngeal hypophysis with a par-

* Received for publication September 28, 1926.

tical with^r that determined by the use of the three levels and the fifteen fields.

GENERAL DESCRIPTION OF THE HYPOPHYSIS

Of the thirty-two specimens of the anencephalic fetus examined, an hypophysis was present in each instance. Such findings are in accordance with the more recent investigations on this monstrosity but in contradiction to the earlier work. It would appear that the earlier work was of a gross nature so that the small amount of hypophyseal tissue which lies on the malformed basis cranii may have been overlooked.

The hypophyseal fossa is usually lacking or at the most only feebly developed. No trace of the clinoid processes or dorsum sella is evident. Occasionally the anterior clinoid processes are slightly developed and so form a narrow fossa for the most anterior portion of the gland. A diaphragma sella is lacking, probably because of the absence of the clinoid processes.

The hypophysis may then be said to lie on a flattened sella turcica covered over by membranes, vascular tissue and in some instances a small amount of brain tissue. Macroscopically it presents a flat triangular shape with the apex directed dorsally. The base of this mass is formed by two lateral spreading portions and the apex of one central mass. Such a shape no doubt is of embryologic significance because it is known that the glandular portion (pars anterior) grows in early fetal life by means of two lateral and one central buds from the ventral side of Rathke's pouch. This form subsequently changes in the course of normal development because the gland soon approximates the shape of the fossa in which it is situated. Macroscopically, the neural elements (pars nervosa) of the gland, when present, appear as an irregularly shaped opaque mass on the dorso-posterior surface of the larger glandular portion. An infundibular stalk is lacking and only a thin cord of membrane remains to attach the gland to the overlying tissues. Microscopically, the hypophysis of the anencephalic fetus presents several striking differences when compared with the gland from the normal fetus. In the first place, the three lobes may not all be present. In only six of the seventeen specimens studied did the hypophysis consist of three lobes. In eleven instances there was no pars nervosa evident. In four of the latter a pars intermedia could be distinguished, while in the remain-

ing seven the pars anterior appeared to comprise the total gland volume.

The extreme vascularity of the gland, particularly of the pars anterior, presents a marked difference from that of the normal. Especially about the periphery of the anterior lobe the sinuses are dilated to such an extent that the parenchyma is reduced to narrow epithelial cords which appear to be surrounded by blood spaces. Toward the center of the pars anterior, the sinuses are usually less dilated with blood.

Trabeculae radiate through the pars anterior and contain numerous blood vessels. Occasionally there is a band of fibrous tissue separating the main bulk of the anterior lobe from the thin epithelial strip which borders the hypophyseal cavity. Likewise the pars intermedia may be separated from the pars nervosa by a thin band of fibrous tissue. The capsule of connective tissue about the gland is well developed and appears to be more extensive than in the normal hypophysis.

RELATIVE AND ABSOLUTE VOLUMES OF THE GLAND AND ITS PARTS

A. *Weight of the total gland.* In Table 1 are given the absolute volumes of seventeen hypophyses and their lobes. It is evident that there is considerable individual variation in the total weight of the gland for fetuses of about the same age. The six observations for fetuses of the fifth to the sixth fetal months have a range of 29.3 to 69.6 mg. and an average of 42.3 mg. The range as observed from six specimens of the sixth to the seventh fetal months is 29.2 to 85.6 mg. with an average of 58.5 mg. Four hypophyses from fetuses of the seventh to the eighth fetal months show a range of 35.0 to 73.8 mg. and an average of 55.1 mg. The intervals from the eighth fetal month to birth are represented by only one observation. The age of the latter was calculated as 10.16 fetal months and the hypophysis weight was found to be 113.2 mg.

B. *Weight of the pars anterior.* Considerable variation is likewise evident in the weights of the pars anterior. For the fifth to the sixth months, the range is 29.3 to 65.4 mg. and the average is 40.9 mg. The weights of this lobe in specimens of the sixth to the seventh fetal months show a range of 28.9 to 85.6 mg. with an average of 57.8 mg. From the seventh to the eighth fetal months, the range is 34.6 to

TABLE 1

Absolute Volume of the Gland and its Parts in Seventeen Specimens of Anencephaly

Case number	Sex	Leg length cm.	Total body length computed cm.	Age in fetal months	Hypophysis weight mg.	Pars anterior weight mg.	Pars intermedia weight mg.	Pars nervosa weight mg.
c9.....	F	10.2	25.34	5.38	38.0	36.6	0.9	0.5
c2.....	M	10.7	26.51	5.56	41.6	40.8	0.8	...
c10.....	F	11.0	27.20	5.66	37.3	37.3
c4-17.....	F	11.4	28.14	5.81	69.6	65.4	2.5	1.7
c23-184.....	F	11.7	28.83	5.93	29.3	29.3
c4.....	F	11.7	28.83	5.93	38.4	36.1	1.3	1.0
c23-183.....	F	12.0	29.53	6.05	72.7	69.5	2.3	0.9
c7.....	F	12.7	31.16	6.32	50.4	50.4
c1.....	F	12.9	31.63	6.39	29.2	28.9	0.3	...
c6.....	M	13.1	32.09	6.47	41.2	40.3	0.9	...
c8.....	F	13.2	32.33	6.51	85.6	85.6
c12.....	F	13.7	33.49	6.72	72.2	72.2
c15.....	M	14.5	35.35	7.05	68.9	68.9
c3.....	F	14.6	35.58	7.08	35.0	34.6	0.4	...
c5.....	F	15.5	37.67	7.47	42.9	41.4	1.5	...
c14.....	F	16.6	40.23	7.95	73.8	71.4	2.0	0.4
c4-18.....	F	21.2	50.93	10.16	113.2	106.3	1.6	5.3

TABLE 2

Relative Volumes of the Lobes of the Anencephalic Hypophysis

Case number	Age in fetal months	Pars anterior	Pars intermedia	Pars nervosa
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
c9.....	5.38	96.33	2.36	1.31
c2.....	5.56	98.08	1.92	...
c4-17.....	5.81	93.97	3.59	2.44
c4.....	5.93	94.01	3.39	2.60
c23-183.....	6.05	95.58	3.14	1.28
c1.....	6.39	98.97	1.03	...
c6.....	6.47	97.82	2.18	...
c3.....	7.08	98.86	1.14	...
c5.....	7.47	96.50	3.50	...
c14.....	7.95	96.75	2.71	0.54
c4-18.....	10.16	93.90	1.41	4.69

71.4 mg. and the average is 54.1 mg. In the one gland of a fetus of 10.16 fetal months the weight of the pars anterior is 106.3 mg.

C. *Weights of the partes intermedia and nervosa.* The absolute volumes of the partes intermedia and nervosa are of very little significance because of the extreme variations. They are, however, of importance for a comparison with the normal weights.

D. *Relative volumes of the parts (lobes).* The relative volumes of the various lobes of the hypophysis are given in Table 2. Because of the limited number of cases and the apparent irregularities in the partes intermedia and nervosa, the relative volumes will be considered without respect to fetal age. The anterior lobe comprises about 93.9 to 100 per cent of the total gland volume. In the eleven instances in which a pars intermedia was present, it is seen to comprise 1.14 to 3.59 per cent of the total gland weight. The pars nervosa which occurs in only six of the seventeen cases varies in relative volume from 0.54 to 4.69 per cent. From the above percentage values it is evident that most of the hypophysis is pars anterior and only a small part is intermediate and posterior lobes. In only one instance does the pars nervosa exceed the pars intermedia in relative as well as absolute volume.

VOLUMETRIC ANALYSIS OF THE PARS ANTERIOR

A. *Relative volume of the blood.* Because of the engorged sinuses of the pars anterior and the prominent trabeculae, it was found necessary to determine the relative amounts of each. This was accomplished by means of the paper-weight method as already described. The results are tabulated in Table 3. The figures for the relative vascularity appear to vary from about 25 to 50 per cent for individual estimations. The average for the fifteen determinations is 38.98 per cent.

B. *Relative volume of the trabeculae.* The relative amounts of trabeculae present in the gland range from about 0.5 per cent to nearly 4 per cent. The average for the series is 1.58 per cent. This figure represents only the larger masses of fibrous tissue in the pars anterior. The finer connective tissue framework is included with the percentage figure for the parenchyma, the average for which is 59.44 per cent of the volume of the pars anterior. The range is approximately 45 to 77 per cent.

It is thus obvious that considerable of the absolute volume of the anterior lobe is due to the presence of blood in the sinuses. From the relative averages cited above, it may be said that approximately two-fifths of this part of the gland is composed of vascular and

TABLE 3

Volumetric Analysis of the Components of the Pars Anterior

Case number	Blood	Trabeculae	Parenchyma and finer fibrous stroma
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
c9.....	38.18	3.98	57.84
c2.....	40.37	0.49	59.14
c4-17.....	43.81	2.02	54.17
c4.....	37.83	1.12	61.05
c23-183.....	36.71	2.34	60.95
c7.....	34.48	1.03	64.49
c1.....	21.56	1.40	77.04
c6.....	46.67	0.75	52.58
c8.....	53.00	2.16	44.84
c12.....	40.05	0.97	58.98
c15.....	37.81	1.59	60.60
c3.....	27.22	1.46	71.32
c5.....	38.93	1.47	59.60
c14.....	36.63	2.00	61.37
c4-18.....	51.46	0.97	47.57
Mean.....	38.98	1.58	59.44

fibrous stroma. The remaining three-fifths is comprised of parenchyma and finer fibrous stroma. Such an analysis is of value in determining the actual amount of glandular tissue which is present. The large amounts of blood apparently influence the volume of the anencephalic hypophysis to a marked degree.

DISCUSSION

The shape of the anencephalic hypophysis is in contrast to the normally developed fetal hypophysis. Apparently the hypophyseal fossa plays an important rôle in determining the form and dimensions of the normal fetal gland. Since this fossa is lacking or at the most only feebly developed in anencephaly, the hypophysis is flat-

tened and spreading. For this reason it is obvious that the flattened surface might permit the gland to keep its earlier fetal shape as it appears to do.

The gland of the normal fetus is known to consist of three lobes and, in addition to the main body of the gland, a pars tuberalis. Likewise the hypophysis of the anencephalic may be comprised of three lobes but not a pars tuberalis. It has been shown that a pars

TABLE 4

A Comparison of the Observed Average Values for the Weight of the Anencephalic Hypophysis with that of the Normal Fetal Hypophysis According to Age in Fetal Months

Age in fetal months	Number of cases	Weight of the anencephalic hypophysis mg.		Number of cases	Weight of the normal fetal hypophysis mg.		Differences in weight between anencephalic and normal fetal hypophyses mg.
		Range	Mean		Range	Mean	
5 to 6	6	29.3-69.6	43.2	8	16.0- 41.3	30.1	+12.2
6 to 7	6	29.2-85.6	58.5	16	30.2- 52.7	42.4	+16.1
7 to 8	4	35.0-73.8	55.2	12	42.4- 62.3	55.5	- 0.3
8 to 9	13	58.2-121.2	81.2	...
9 to 10	12	78.5-130.1	95.6	...
10.16	1	113.2	113.2	1	112.6	112.6	+ 0.6

nervosa occurred in only six of the seventeen cases. The anterior lobe may then be said to form the main bulk of the hypophysis. The pars intermedia of this abnormal gland may or may not be present. The residual lumen (hypophyseal cavity) likewise is not always in evidence. The lobes of the hypophysis may appear qualitatively similar to the normal (with the exception of vascularity and fibrous tissue of the pars anterior) but vary considerably in quantity both relative and absolute.

Tables 4 and 5 illustrate by a comparison of observed averages the absolute volumes of the normal and abnormal gland. The total weight of the abnormal hypophysis for the fifth to the sixth fetal months exceeds the weight of the normal gland by 12.2 mg. When the average weight is corrected for blood in the pars anterior it checks with the corrected weight value for the normal. The average weight for the sixth to the seventh fetal months is greater than the normal by 16.1 mg. When these averages are corrected for vascular-

ity, the anencephalic exceeds the normal weight by 0.65 mg. The absolute averages for the seventh to the eighth fetal months are

TABLE 5

A Comparison of the Average Total Weight of the Anencephalic Hypophysis with the Normal Fetal Hypophysis after each has been Corrected for Vascularity

Age in fetal months	Number of cases	Observed average weights of the anencephalic hypophysis mg.		Number of cases	Observed average weights of the normal hypophysis mg.		Differences between the average weights as corrected for vascularity of the anencephalic and normal fetal hypophyses mg.
		Uncorrected	Corrected		Uncorrected	Corrected	
5 to 6	6	42.3	26.35	8	30.10	26.35	± 0.00
6 to 7	6	58.5	36.10	16	42.4	35.45	+ 0.65
7 to 8	4	55.2	34.20	12	55.5	47.77	-13.57
8 to 9	13	81.2	67.89
9 to 10	12	95.6	79.93
10.16	1	113.2	71.80	1	112.6	98.12	-26.32

TABLE 6

Relative Volume of the Blood in the Pars anterior of the Normal Fetal Hypophysis

Necropsy number	Total body length cm.	Relative volume of blood per cent	Relative volume of remainder of pars anterior per cent
24-165	28.0	17.97	82.03
24-25	31.0	12.15	87.85
24-24	38.0	9.86	90.14
23-849	39.0	26.87	73.12
22-323	45.0	10.74	89.26
24-795	45.5	18.82	81.18
24-72	46.0	16.71	83.29
23-224	49.0	12.12	87.88
1746	51.5	21.36	78.64
23-408	55.0	16.41	83.59
23-535	60.0	17.13	82.87
Mean		16.40	83.60

about the same. The corrected values show the normal to be more than the anencephalic hypophysis in volume by 13.57 mg. Since there are no specimens between the age limits of the eighth and the

tenth fetal months, a comparison is not justified. The weight of the postmature specimen is about the same when compared with a normal of the same age. After correction for vascularity the normal exceeds the total weight of the gland in anencephaly by 26.3 mg.

It is obvious that if the amount of blood in the pars anterior be considered, the gland of the abnormal fetus usually is less in weight than the normal from a fetus of corresponding age. The same conclusion holds for the weights of the anterior lobes of normal and abnormal fetuses. The average relative amount of blood present in the normal fetal hypophysis is 16.4 per cent for eleven determinations. This figure is nearly two and one-half times less than the average for the pars anterior of the anencephalic gland.

SUMMARY

The results obtained by this study may be summarized as follows:

1. An hypophysis is present in anencephalic fetuses.
2. It is extremely variable in weight. If the weight is corrected for vascularity it is usually less than the weight of the normal fetal hypophysis which has also been corrected.
3. The pars nervosa is lacking in the majority of cases. When present its relative and absolute volumes are considerably less than those of the normal.
4. The pars intermedia is variable in both occurrence and volume. It may be present in a gland in which there is no pars nervosa in evidence.
5. The pars anterior comprises most of the gland volume and apparently the total gland volume in some instances.
6. The average relative volume of blood present in the anterior lobe is about 39 per cent of the volume of that lobe.

The writer wishes to acknowledge his indebtedness to Professors R. E. Scammon and A. T. Rasmussen for their many valuable suggestions during the course of this study.

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SYNGENESIOTRANSPLANTATION IN THE GUINEA-PIG *

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In a former paper we compared syngenesiotransplantation with homoiotransplantation and furthermore we compared with each the effect of exchange of pieces of tissues or organs between brothers, or of the transplantation of pieces from parent to children or from children to parents.¹ In general, syngenesiotransplantations behaved in a way intermediate between auto- and homoiotransplantation; however, there were considerable differences between the syngenesiotransplantations in individual cases. In addition there seemed to be differences between the following types of transplantation: I, brother to brother; II, children to parents; and III, parents to children. Type I gave the best results, type II gave the worst results and the results in type III were intermediate between those in type I and type II. However, the number of transplantations from parents to children and from children to parents was limited and it was unsafe to draw conclusions from a small number of experiments. We therefore decided to add to these various series of transplantations other experiments in order to obtain more definite data concerning syngenesiotransplantation. We not only increased the number of experiments, but we also extended them by including transplantation of tissues of grandchildren to grandparents and *vice versa*, and by increasing the number of organs used for transplantation.

In order to be able to summarize our results and to compare them with the results of homoiotransplantation, it will be necessary to use a method, similar to the one applied by us previously on various occasions, of grading the conditions found in the transplant. It will be of value to know the grades in the individual transplantations as well as the average grade of the various kinds of transplantations. The former indicate the degree of variations in syngenesiotransplantations as compared with those found in other kinds of transplantations. However, there is one variable factor which complicates the

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various periods from fifteen to forty days after operation. Of these thirty cases, six behaved like autotransplants or approached autotransplants; twelve showed the character of syngenesiotransplants and twelve cases were like homoiotransplants or approached homoiotransplants. The individual grades were as follows: 15 days: 3 (?); 17 days: 5, 6, 3.25, 3 (?); 18 days: 5.5, 6, 3 (?), 4; 20 days: 2, 4, 6; 25 days: 1; 26 days: 5; 28 days: 4, 2; 29 days: 5; 30 days: 4, 3, 2, 5.5; 33 days: 5; 35 days: 1, 4.75, 2, 2, 3.25, 1; 40 days: 3.75, 6. The average of these thirty pieces is 3.7. As illustrations, the following abstracts of records may be cited.

(a) 40 days. *Thyroid*. Many acini with colloid preserved, but separated by lymphocytes and fibrillar connective tissue. Center of transplant filled with lymphocytes. Over a wide area acini have been destroyed. *Cartilage*. Well preserved and surrounded partly by areolar tissue and partly by fibrous tissue. Much lymphocytic infiltration in areolar as well as in fibrous tissue. Grade 3.75.

(b) 40 days. *Thyroid*. Excellent preservation; acini with low to medium-sized epithelium, close together with solid, retracted colloid. In center of transplant some areolar and connective tissue and a small collection of lymphocytes. *Cartilage*. Well preserved and surrounded by areolar tissue and some fine fibrillar connective tissue; no lymphocytes. Grade 6.

(c) 33 days. Transplants taken out soon after death of animal. *Thyroid*. Almost like autotransplant, but increased lymphocytic infiltration at points in periphery and in places in center, from here penetrating between some acini and surrounding them. Lymphocytic masses enter also into epithelium of some acini. Some lymph vessels filled with lymphocytes. Some acini in process of destruction. The center of transplant is composed of areolar and some dense fibrous tissue; it is surrounded by a ring of thyroid tissue. With the lymphocytes, connective tissue cells penetrate between the acini. In this case the transplant had at first the character of an autotransplant; secondarily some connective tissue cells with considerable collections of lymphocytes penetrated beneath and between the acini and separated them from each other. *Cartilage*. Much cellular cartilage. The transplant is surrounded by areolar tissue, the septa of which are somewhat thickened. There is a slight new formation of connective tissue, with a few lymphocytes. Grade 5.

(d) 30 days. *Thyroid*. Ring of well preserved acini. In center large vessels surrounded by edematous connective tissue. Medium-sized acinus cells. Acini close together and with colloid; in other places widely separated by lymphocytes. Frequent mitoses in acinus and connective tissue cells. In places also compression and destruction of acini and acini without colloid, though in certain areas many acini may be left. Some acini filled with cells. Lymph vessels in center filled with lymphocytes. Grade 4.

(e) 26 days. *Thyroid and parathyroid*. Very well preserved. Thyroid consists of large masses of acini which are close together and have solid colloid. Relatively little connective tissue in center of transplant, but much lymphocytic infiltration here and at one pole of the periphery. At numerous points lymphocytes begin to penetrate between acini. In center also lymph vessels filled with lymphocytes. Around thyroid and parathyroid some groups of lymphocytes, which in various places just begin to infiltrate thyroid. *Ovary*. Good primordial, small and medium-sized follicles with follicular cavities. Good ova in center. Mitoses in granulosa of follicles. No follicles with degeneration of granulosa; therefore no dividing egg. In some small follicles without cavity eggs are being destroyed. In center large areas of necrotic tissue organized by loose connective tissue. Atretic yellow bodies. Cavities of atretic follicles. Wide medullary canals; in places some marked lymphocytic infiltration in periphery. Lymphocytes are also in cortex and encroach upon some primordial follicles, but the latter are, on the whole, well preserved. No germ epithelium left. In periphery, part of corpus luteum is preserved, but it is somewhat vacuolar and moderately infiltrated with lymphocytes. Some polymorphonuclear leucocytes in cortex of ovary among the lymphocytes. On the whole, considerable lymphocytic infiltration; the lymphocytes are found in groups here and there and also almost in a continuous ring. The lymphocytes cannot penetrate to periphery of central fibrous areas. Grade 5.

(f) 20 days. *Thyroid*. In the center which is small are large vessels and loose edematous connective tissue. Excellent ring of acini with much colloid. Solid colloid retracted. Acini close together, well vascularized. Some acinus cells take up blood pigment. A small number of lymphocytes around central vessels. No lymphocytes migrate through thyroid. Grade 6.

These six cases differ from those which are characteristic of

homoiotransplants. The centers show the structure of autotransplants, indicating that in the first period following transplantation the host tissue behaved toward the transplant as if it had been autotransplanted tissue; but subsequently the lymphocytes are attracted by the transplant, and they invade and partly destroy it. At the same time a moderate new formation of connective tissue takes place. The lymphocytes migrate into the living, actively metabolizing, and not into dying thyroid. In other cases, the syngenesio-toxins are produced in so great a dilution, that the transplant acts like an autotransplant as late as forty days after transplantation. Still other transplants resemble homoiotransplants, behaving similarly to the transplants described in our preceding papers on homoiotransplantation. We see that the condition of the cartilage transplant is parallel to that of the thyroid transplant, although the reactions against the cartilage and fat transplant are less pronounced than those against the thyroid. The transplanted ovary is well preserved; the follicles develop to a certain size and contain normal ova. Lymphocytes invade also the ovary, but the infiltration does not become very intense. In two cases host and donor were the offspring of parents one of which was smooth haired, while the other one was curly haired. The grades in these two cases were 4 and 2.

Series II. Transplantations from children to parents. In some cases only thyroid, in other cases both thyroid and cartilage were used for transplantation. Altogether 104 transplantations were made in this series. Between seven and sixteen days (inclusive) after transplantation, thirteen cases. *11 days*: approaching homoio-character. *12 days*: (a) decided syngenesio-homoio-character; (b) syngenesio-character. *14 days*: (a) approaching homoio-character; (b) through infection the greater part of thyroid destroyed. *15 days*: (a) homoio-; (b) syngenesio-; (c) homoio-; (d) homoio-; (e) homoio-character. *16 days*: homoio-character, with intense lymphocytic infiltration. *17 days*: 8 cases; *18 days*: 9 cases; *19 days*: 19 cases; *20 days*: 22 cases; *21 days*: 6 cases; *22 days*: 4 cases; *24 days*: 2 cases; *25 days*: 8 cases; *26 days*: 2 cases; *27 days*: 2 cases; *28 days*: 3 cases; *29 days*: 2 cases; *31 days*: 4 cases.

If we omit the case taken out and examined seven days after transplantation and one other instance in which some special conditions were present, we may classify the remaining cases as follows: Approaching auto-character: 6 cases. Good syngenesio-character: 6

cases. Syngenesio-character: 10 cases. Decided syngenesio-character: 6 cases. Syngenesio-homoio-character: 24 cases. Homoio- or approaching homoio-character: 50 cases; some of these were doubtful, no thyroid being found. Altogether favorable transplants (syngenesio- to approaching auto-character): 22 cases. Unfavorable cases (homoio- to bad syngenesio-character): 80 cases.

If we grade the cases from seventeen days to thirty-one days (inclusive) after transplantation, we find the average grade of the ninety-one cases is 2.91, which is decidedly below that obtained in the brother to brother transplantations. The individual grades vary between 1 and 6.

Grades at 17 days: 3, 3.5, 3.5, 2, 5, 5, 3.5. Grades at 18 days: 2.5, 2.25, 6, 3, 4.5, 4.5, 4.5, 1, 4.5. Grades at 19 days: 5.5, 5.75, 4, 4, 3.5, 2, 1 (?), 3, 3.5, 1 (?), 1.5, 3.5, 4, 3.5, 4.5, 1 (?), 1 (?), 3.25, 3.25. Grades at 20 days: 3, 3, 1, 1.5, 2, 4, 3, 5.5, 6, 1.5, 4.5, 3, 4, 4, 3.5, 1, 4, 1 (?), 1 (?), 4.5, 3.5. Grades at 21 days: 1, 2, 3.75, 3, 1, 3. Grades at 22 days: 5.75, 3, 2.5, 4. Grades at 24 days: *2, *2. Grades at 25 days: *1, *2, *1, *2, *2 (?), 1 (?), 6, 3. Grades at 26 days: *1, 2. Grades at 27 days: *1, *1. Grades at 28 days: *3 (?), 5, 1. Grades at 29 days: 2.5, 2.5. Grades at 31 days: *3, 3 (?), 1.5, 1.5.

In those cases in which the grade has an asterisk, the parents showed more marked differences than in the rest of the cases, one parent having smooth and the other rough hair. Twelve such transplantations were made, the average grade being 1.7, which is considerably below the average grade in this series. This indicates that the reaction in the parents against the tissues of a child carrying these strange individuality differentials is greater than that against the tissues of a child, both parents of which had smooth hair. On the other hand, in a number of instances in which very good results varying between auto- and syngenesio-character were obtained, there is some indication that the parents were related to each other. Thus in four out of six cases in which the result approached an auto-condition, the parents were in all probability related to each other. In the two remaining cases, this was not certain. Among the sixteen cases in which a syngenesio-reaction was obtained, in seven cases there was a definite indication or a suggestion that the parents were related to each other. Thus the type of reaction obtained in transplantation from children to parents depends upon the relationships of the individuality differentials of host and donor.

Twenty-nine experiments were arranged in the following manner. In certain cases a thyroid lobe, with or without a piece of xiphoid cartilage, of a child was transplanted to the father and to the mother; in other cases a thyroid lobe and piece of cartilage were transplanted from a child to one side of mother or father and from a second child to the other side of the same parent. The pieces were taken out at the same time for examination. Our aim was to determine how often the tissues of both parents would behave in the same way toward the tissues of the same child and how often they would behave differently; also how often one parent would behave in a like manner or differently to the tissues of two children.

The results were as follows: Two of these twenty-nine cases have to be discarded because in one host the transplant was not found. In seventeen cases the transplants of a pair behave in the same way or in almost the same way; in all except two of these pairs a homoio-reaction or a condition approaching it was obtained. In one case both members of a pair behave like autotransplants and in another case like decided syngenesiotransplants. In four pairs the two members behaved differently. In two cases one member of each pair showed an auto-reaction and the other a reaction standing between syngenesio- and homoio-reaction; in the other two cases a homoio-reaction was combined with a good syngenesio-reaction. In six cases the pairs differed, but the difference was less marked than in the former two cases. Considering the preponderance of homoio-reactions in this series we must expect to find in many cases homoio-reaction in both transplants. We may then conclude that in a number of cases the two partners of a pair behave differently, that the individuality differentials of brothers differ or that the individuality differential of a child has a greater similarity to the individuality differential of one parent than to that of the other parent. While in many cases in this series the result corresponds to or approaches a homoio-reaction, still it differs from that in the series of homoio-transplantations, since there are in the latter exclusively homoio-reactions or reactions approaching this condition and in the former a considerable number of auto- and syngenesiotransplantations are added to the homoio-reactions. The average grade is therefore higher in the present series. We may cite some examples of transplantations from children to parents. (a) 18 days. From 12-13 days old child to mother. *Thyroid*. Much dense fibrous tissue, but no

wide vessels in center. Very incomplete ring of acini; almost two-thirds of thyroid is destroyed. Acini separated by much diffuse lymphocytic infiltration; mitoses in acini which are attacked by lymphocytes. In large majority of acini the colloid destroyed by phagocytes, but some plates of colloid left. Grade 2.75.

(b) 18 days. From child to father. *Thyroid*. In center of transplant some dense fibrous tissue, but in the periphery of center edematous connective tissue and large vessels penetrating through thyroid ring into center. Good ring of acini containing colloid and lying in close approximation to each other. The *parathyroid* transplant also well preserved; some loose connective tissue in center. Lymphocytic infiltration lacking. Grade 6.

(c) 19 days. From child to father. *Thyroid*. Large lymph and blood capillaries in periphery of center. Rather intense lymphocytic infiltration in center, from here penetrating into thyroid ring. An area of well preserved acini, close together and containing well formed colloid; but part of thyroid destroyed. Grade 3.75.

(d) 25 days. From child to father. Mother was Abyssinian, father smooth haired. Child has some characters from father and some from mother. *Thyroid*. Only fibrous tissue, with blood pigment and lymphocytic infiltration, found; no thyroid tissue left. *Cartilage*. Fibrous tissue and very marked lymphocytic infiltration surrounding cartilage. End of cartilage consists of cartilage cells with very little hyaline substance separating them; here lymphocytes deeply invade cartilage and injure it.

Series III. Transplantations from parents to children. In this series thirty-three transplantations were made; two transplants were examined as early as seven and almost nine days after transplantation; the other pieces were taken out after periods varying between ten and thirty-two days. The results in all except the first two cases were as follows: auto-reaction in 4 cases (10, 14, 17 and 20 days after transplantation); syngenesio-reaction in 2 cases (18 and 20 days); marked syngenesio-reaction in 2 cases (20 and 30 days); homoio-reactions or reactions approaching these in 23 cases (14 to 32 days). In one-sixth of the cases auto- or syngenesio-reactions were obtained. The grades are as follows: 10 days: 6; 14 days: 4, 2.5 (?), 6; 17 days: 6; 18 days: 1 (?), 2.5, 4.5; 20 days: 5.75, 1.5, 3.5, 4; 21 days: *1.5, *2; 25 days: *1, *1, *1.5, *1, *2, *2, *1 (?); 29 days: 1, 1; 30 days: *3.25, *1.5, *3, *2.75, 1.5, 2.5; 32 days: 3.25, 1.5. The

average for the series is 2.6. In thirteen cases, marked with an asterisk, where one parent was smooth and the other rough haired, the average grade is 1.8. The reaction is therefore stronger in these cases and the injury of the transplant greater. This is the same result which we obtained in the transplantation from children to parents. In nine cases in this series, pairs were used for transplantation similar to those we discussed in the transplantation from children to parents. In some cases the thyroid lobe of one side and piece of cartilage were transplanted to one child and the other lobe and piece of cartilage to a second child; or in other cases a thyroid lobe and a piece of cartilage from one parent were transplanted into the right side and from the other parent into the left side of the same child. In all cases the paired transplants behaved in the same or in a similar way; in seven instances a homoio-reaction was obtained in both members of the pair. This was to have been expected considering the large number of homoio-reactions or reactions approaching them in this series.

The following examples may be cited: (a) 20 days. *Thyroid*. Incomplete ring of acini. Much lymphocytic infiltration in center and between acini; also increased fibrillar connective tissue between acini; in some cases lymphocytes enter the colloid in the center of acini and destroy it. In places acini are still close together. *Cartilage*. The transplant surrounded by loose areolar connective and fat tissue. There is some lymphocytic infiltration in the fat tissue around cartilage and it extends farther out into fat tissue. At both ends of the cartilage there is some regenerated cartilage. Grade 4.

(b) 25 days. Father smooth, agouti; mother, rough haired, red; child, rough haired, agouti and red. From father to child. *Thyroid*. Instead of transplant only hyaline fibrous material with some lymphocytes is found; no thyroid left. *Cartilage*. Partly alive and partly necrotic; especially where it is thick we find in the center some pyknosis and loose cells. The piece is surrounded by fibrous tissue which includes some remaining fat cells. Very moderate lymphocytic infiltration around cartilage. Grade 1.

Series IV. Transplantation from grandchildren to grandparents. This series as well as the one following is smaller than the preceding series of syngenesiotransplantations. Thirteen experiments were carried out; the examination took place from the twentieth to the twenty-seventh day after operation. In two of these cases the result

was an auto-reaction, while in the eleven other cases the results approached or were definite homoio-reactions. In some instances only cartilage was transplanted and then the cases were difficult to grade since, as we have stated on previous occasions, cartilage is a less delicate indicator than thyroid of the reaction on the part of the host toward the transplant. With this reservation we give the following list of grades which we obtained in this series: 6, 5.75, 3, 2.75, 2.25, 2 in six cases, 1.5, 1. While these grades are relatively low, still they are better than those obtained in homoiotransplantations.

In four cases pairs were examined in the same way as in the preceding series; in three of these pairs the results were the same, the grades corresponding to or approaching a homoio-reaction. In one case, a splitting of characters took place. The grandfather was an Angora guinea-pig, and the grandmother was smooth; the grandchild was an Angora and resembled therefore the grandfather. In this case the tissue that had been transplanted into the grandfather resembled an autotransplant, while the tissue that had been transplanted into the grandmother resembled a homoiotransplant. The individuality differentials in this instance corresponded to the similarities of appearance and race characteristics. If we exchange tissues between hybrids and ancestors belonging to different races, an auto-reaction should be obtained in a certain proportion of cases.

Series V. Transplantation from grandparents to grandchildren. In this series seventeen transplantations were made; the pieces were taken out for examination from twenty to twenty-seven days after operation. The results were as follows: An auto-reaction was obtained in two cases, a syngenesio-reaction in five cases and a reaction approaching or equal to a homoio-reaction in ten cases. Grades: *20 days*: 2, 4.5, 5.5, 2, and in one case, probably of homoio-character, it was impossible to determine the grade; *22 days*: 1.5, 1, 1, 4.5, 4.5; *24 days*: 4, 2, 2.5; *25 days*: 1, 1.5; *27 days*: 4.75, 6. The average of the grades is 3. The average results are better than in homoiotransplantations owing to the number of auto- and syngenesio-reactions obtained. In seven instances, pairs were compared. The results in the members of the same group were usually similar. In four pairs all the pieces showed or approached homoio-character. In one pair both transplants were very well preserved and called forth only a very slight reaction. In one pair the transplants from one member showed an auto-, and from the other member, a syngenesio-reaction;

in this case some difference in the behavior of the two partners was therefore observed.

CONCLUSIONS

The reactions against syngenesiotransplantations in the guinea-pig are intermediate in intensity between auto- and homoio-reactions. However, this statement is correct only so far as the averages are concerned; the individual reactions may either approach or reach auto-reactions, on the one hand, and on the other, homoio-reactions. There are, however, typical intermediate reactions which we may designate as syngenesio-reactions in the strict sense. They occur when the individuality differentials between host and transplant are not identical but are so much related to each other that toxins develop late and not at a very early stage after transplantation. It seems that some time must elapse before a relatively slight incompatibility between the differentials permits a concentration of injurious substances sufficient to cause the disturbances. Thus in the first period after transplantation (in the case of thyroid) between the sixth and twelfth day, when the general structure of the transplant is laid down, the absence of a sufficient amount of individuality toxins allows the development of auto-structure; the connective tissue is, to a large extent, loose in the center and small in amount; the vascularization is good and the thyroid ring develops well; connective tissue is not increased between the acini and lymphocytes are absent. Then gradually when the concentration of toxins becomes sufficiently strong, lymphocytes appear, fill the lymph vessels and invade the transplant. These cells collect especially in large numbers at the inner edge of the thyroid ring; from here they penetrate between and also into the acini, which are still perfectly well preserved, and help to destroy them. There may now probably develop secondarily some connective tissue between the acini. Between this type of reaction which we observed in a number of cases, and the typical homoio-reaction on the one hand, and the auto-reaction on the other hand, all kinds of intermediate types exist. When tissues other than thyroid are transplanted, conditions are similar; but the reactions of the host against cartilage are not so sharply demarcated from each other as they are against thyroid. In the case of the cartilage transplant the amount of fibrous tissue development in the fat and areolar tissue, and the degree of lympho-

cytic reaction in the fat tissue and around the cartilage are indicators of the intensity of the reaction. There is, on the whole, a good agreement between the various reactions if different kinds of tissues are transplanted simultaneously from the one donor into the same host. There are then two ways in which the intermediate average between homoio- and auto-reaction is brought about in the case of syngenesiotransplantations, namely, (1) in the first place, reactions approaching both auto- and homoio-character occur in different cases and as far as the average result is concerned compensate each other, and (2) in a number of host-transplant combinations actual intermediate reactions take place. The averages in the different types of syngenesio-reactions were as follows:

1. Brother to brother transplantation, 3.7.
2. Children to parents transplantation, 2.91.
3. Parents to children transplantation, 2.60.
4. Grandparents to grandchildren transplantation, 3.
5. Grandchildren to grandparents transplantation, 2.6.

The brother to brother transplantations give the best results. The transplantations of parent to children, grandparents to grandchildren and *vice versa* give results not so good as those from brother to brother; but all are intermediate in character, although the averages of the transplantation taking place between members of different generations are distinctly nearer the homoio-type than the auto-type. If we assume that the individuality differentials are composed of a considerable number of factors, perhaps a number corresponding to the number of the chromosomes except the sex chromosomes, and that the presence in the transplant of factors strange to the host causes indirectly the reaction against the transplant, then we must expect that brother to brother transplants should on the average call forth the least intense reactions. As to the transplantations from parent (grandparents) to children (grandchildren) they should both lead to more marked reactions. In one case the total average was somewhat better in the transplantations from children to parents than in the reverse transplantations from parents to children. On the other hand, the total average was slightly better in the series in which pieces were transplanted from grandparents to grandchildren than in the reciprocal series. These differences are probably to a large extent due to the composition of the families used for transplantations. Exchange of tissues between

different generations should give poorer results if the individuals used are not at all related or if they belong to different races, than those obtained in families where some inbreeding has taken place. This probably explains the fact that we obtained the lowest average grade in transplantations which were made from parents to children; in this series there were a number of families where one of the parents had smooth, the other rough hair.

These results agree with our former investigations in that they confirm the intermediate position of the average grade for syngenesiotransplants as well as the intermediate character of the syngenesio-reaction proper. They confirm also our conception of the mode of syngenesio-reaction and the part connective tissue, blood vessels and lymphocytes play in this reaction. But at the same time we carried our analysis further by experimenting on a much larger scale than we did previously; we showed the relative frequency of the various types of reactions in each kind of syngenesiotransplantation. Furthermore, we found reasons for assuming that the character of the parents (the degree of their relationship or race differences existing between them) influences the results of syngenesiotransplantation. While we thus found, as in our previous investigations, that brother to brother transplantations have the highest average grade, we did not obtain very far-going differences between parents to children and children to parents transplantations. Such differences as we observed were probably, at least in a measure, due to genetic relationships or differences between the parents. Thus we supplement and correct some of our previous figures which were in part based on a limited number of experiments.

If we compare the individuality differentials of both parents with the individuality differential of their child, or if we compare the individuality differentials of two brothers with that of one of their parents, we find that the members of a pair may behave differently; but in the majority of cases they behave similarly. This might be expected if we consider the fact that in so many cases homoio-reactions obtain; according to the law of probabilities a considerable number of individuality differentials of pairs should be similar under these circumstances.

SUMMARY

1. Syngenesio-reactions in the guinea-pig have a position intermediate in character between homoio- and auto-reactions. This intermediate character is due to the variations in individual reactions which approach auto-reactions, on the one hand, and homoio-reactions, on the other hand, as well as to the existence of a real intermediate syngenesio-reaction.

2. The typical intermediate syngenesio-reaction resembles the auto-reaction as far as the behavior of connective tissue and vessels is concerned. It differs from the auto-reaction in as much as subsequently lymphocytic masses invade the transplant.

3. Brother to brother transplantations resemble autotransplantations to a higher degree than parent to children or the reciprocal transplantations. The grade of transplantations from parent to children does not seem to be significantly higher than the grade for the reciprocal transplantations.

4. Transplantations between grandparents and grandchildren have average grades similar to those of transplantations between parents and children.

5. Relationship or dissimilarity (race differences) between the two parents seems to influence the intensity of reactions in the exchange of tissues between the various members of families.

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SYNGENESIOTRANSPLANTATION IN THE RAT *

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In the preceding paper of this series we discussed the results obtained in syngenesiotransplantation in the guinea-pig. We have carried out corresponding experiments in the rat and we shall discuss these in the present paper, thus extending previous experiments in this species, in which multiple syngenesiotransplantations of various organs were made.

In order to summarize our findings we shall use the same set of grades which we defined in our preceding paper.

SERIES I. TRANSPLANTATION FROM BROTHER TO BROTHER

(1) *19 to 25 days after transplantation.* Thirteen experiments in which pieces often of several organs were transplanted into the same animal. In ten cases the reactions approached or were identical with auto-reactions; in the three remaining cases the reactions were not so good, but still were better than homoio-reactions. Grade 6 in 5 cases; grade 5.75 in 3 cases; grade 5.5 in 1 case; grade 5.25 in 1 case; grade 4.5, grade 3.25 and grade 3, each in 1 case. Average grade 5.29.

(2) *26 to 35 days (incl.) after transplantation.* Twenty-three experiments. In eleven cases the reactions approached or were identical with auto-reactions; in twelve cases the reactions ranged between those characteristic of syngenesio- and of homoiotransplantations. In various cases there was a very moderate or strong lymphocytic reaction present. Grade 6 in 3 cases; grades 5.75 and 5.50 in 4 cases; grades 5.25 and 5 in 6 cases; grade 4 in 3 cases; grades 3.75, 3.50 and 3 in 3 cases; grades 2.75, 2.50 and 2.25 in 4 cases. Average grade 4.49. In this period the intensity of the lymphocytic reaction increases somewhat and on the whole the more so, the later the period of examination. The lymphocytic infiltration can be present without a concomitant connective tissue reaction.

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(3) *40 to 60 days after transplantation.* Sixteen experiments. In eight cases the reactions approached or were identical with an auto-reaction. Moderate lymphocytic reaction in two cases; much lymphocytic reaction in four cases; homoio-reaction in two cases. Grade 6 in 5 cases; grade 5.50 in 2 cases; grade 5 in 1 case; grade 4.5 in 2 cases; grades 3.75 and 3.25 in 3 cases; grade 2.75 in 1 case; grades 1.5 and 1 in 2 cases. Average grade 4.41.

If we compare the average reactions in different periods we find between 19 and 25 days, grade 5.29; 25 and 35 days, grade 4.49; and between 40 and 60 days, grade 4.41. The average grade of the fifty-two experiments (examination between 19 and 60 days) is 4.66.

In twenty-five additional experiments the pieces were taken out at earlier periods, namely between eight and seventeen days. It is difficult, especially between the eighth and fourteenth days to assign grades to the reactions; but if we attempt a grading at this early period, we may summarize the reactions as follows: grade 6 in 9 cases; grades 5.5 and 5 in 4 cases; grades 4.5 and 4.25 in 5 cases; grade 4 in 3 cases; grade 3 in 2 cases; grades 2.5 and 2 in 2 cases. The average grade of this group is 4.8.

In order to illustrate these figures we shall select especially cases in which a syngenesio-reaction was obtained, and in which, in addition to thyroid or cartilage, ovarian tissue or uterus and tube was transplanted.

(a) *19 days. Ovaries.* Large and small well preserved follicles with ova. There were well formed corpora lutea and among them new corpora lutea as well as a retrogressing corpus luteum; also primordial follicles with eggs were seen and adjoining them small collections of similar cells, but without eggs. Thick interstitial tissue in which large, yellow-stained cells were found. Tubes and uterine tissue, epithelial as well as muscle tissue, were well preserved. Grade 6.

(b) *19 days.* The structure of thyroid and cartilage corresponds to an auto-reaction, but in the thyroid transplant there are in a few places considerable collections of lymphocytes around vessels. In the cartilage transplant there are in the fat tissue a few small collections of lymphocytes, also some lymph vessels containing these cells. The number of invading lymphocytes is greater in the thyroid than in the cartilage transplant. The perichondrium produces a new plate of cartilage. Grade 5.5.

(c) 24 days. Good thyroid transplant; acini with medium-sized epithelium and good solid retracted colloid. In the center some ducts and cell nests. Through solution of cell masses, cavities are produced in these cell nests. Around latter and around some central vessels there is a slight lymphocytic infiltration. Also at one pole of the thyroid there is a moderate mass of lymphocytes. Fibrous tissue surrounds large vessels. Well preserved parathyroid transplant. Grade 5.25.

(d) 25 days. Thyroid transplant well preserved. In center some increase in fibrous tissue, but also a few fat cells. Transplanted artery preserved. In various places there are collections of lymphocytes in periphery and in center of transplant. Also individual acini are occasionally separated by lymphocytes. While collections of these cells are frequent, there are wide areas without lymphocytic infiltration but with well preserved acini containing solid colloid. In cartilage transplant much less lymphocytic infiltration than in thyroid. There is a large amount of areolar tissue around cartilage and some slight collections of lymphocytes are found in it; none of these cells in fibrous tissue around areolar tissue. Over wide areas the cartilage is necrotic. Separated from necrotic cartilage through necrotic fibrous tissue there are found sheets of new, living cartilage produced through perichondrial proliferation; this is observed in places on both sides of the cartilage, although not everywhere. Grade 4.5.

(e) 25 days. In thyroid transplant acini are almost without colloid and without lumen. The acini are compressed and have high epithelium. Some loose, partly necrotic connective tissue in center; connective tissue grows also around acini. Large vessels grow into the center. Lymphocytes penetrate the acini and destroy them; but a great number of compressed acini are preserved. Of the uterus transplant only fat and fibrous tissue, with much lymphocytic infiltration, is found. In the transplant of the ovary there are well preserved primordial follicles and eggs and well preserved further developed follicles also with ova. In the granulosa many mitoses. Many atretic follicles and epithelial ducts. Some remnants of interstitial gland. Mitoses in germ epithelium cells; this layer of cells forms a cyst. Lymphocytic infiltration in ovarian stroma; some lymphocytes in granulosa epithelium and also in interstitial tissue; lymph vessels filled with these cells. There is a correspondence in behavior of thyroid and ovary transplants. Grade 3.

(f) *30 days*. Thyroid almost like autotransplant. A little fibrous tissue in center; well formed acini with colloid. Some loose connective tissue and blood pigment cells in center; large vessels here also; several small lymph vessels filled with lymphocytes. A few of the latter cells seen between, and in places entering, the acini. At one point outside of the thyroid rather dense collection of lymphocytes. Also some diffuse lymphocytic infiltration in fibrous tissue here and there in center. Mitoses in acinus cells. Well preserved parathyroid with a few lymphocytes in parathyroid tissue. The structure of the thyroid transplant is that of an autotransplant. Adjoining the cartilage transplant there is a large amount of fat tissue. Cartilage and perichondrium well preserved; where former has been injured, latter proliferates. Muscle tissue that attaches to cartilage, in good condition; occasionally in fat tissue, small collection of lymphocytes. Grade 5.25.

(g) *30 days*. Beginning pregnancy in host. Well preserved thyroid ring, colloid in acini. Some loose connective tissue and fairly marked lymphocytic infiltration in center; lymph vessels filled with lymphocytes. Also some diffuse and some more moderate lymphocytic infiltration in parathyroid. Very much lymphocytic infiltration in connective tissue and perichondrium around cartilage. Grade 4.

(h) *30 days*. Thyroid and parathyroid transplants well preserved; large acini with good colloid. Little connective tissue, some fat tissue and good vascularization in center. Lymph vessels in center filled with lymphocytes. Some localized lymphocytic collection in thyroid. Lymphocytes begin to infiltrate the transplant without any preceding connective tissue changes. Transplanted uterus and Fallopian tubes with well preserved epithelium, connective tissue and musculature. In the transplanted ovary, primordial follicles with eggs small and large and well developed follicles with ova. In some follicles degeneration of granulosa. Medullary ducts. Necrotic corpus luteum with yellow pigment. Interstitial gland. Around foreign body giant cells usually some lymphocytic collection. On the whole very slight lymphocytic infiltration in ovary. Grade 5.25.

(j) *30 days*. Thyroid transplant, with intense lymphocytic infiltration, appears almost like a lymph gland. Only here and there isolated small nests of acini are left. In the center there is somewhat loose connective tissue with moderate lymphocytic infiltration. A

number of acini without colloid and compressed; lymphocytes are around and in them. Also in the surrounding fat tissue and in the parathyroid there is some lymphocytic infiltration. In a short time probably the whole transplant would have been destroyed. Uterine epithelium, mucosa and muscularis preserved; there is some lymphocytic infiltration in the uterus proper, but the most marked infiltration is in the periuterine and in the fat tissue and here it is extreme; the lymph vessels are studded with lymphocytes. In the ovarian transplant a large germ epithelium cyst and interstitial tissue are visible. The greater part of lymphocytic infiltration is around the large lymph and blood vessels. Grade 3.

(*k*) 35 days. Thyroid transplant with intense lymphocytic infiltration. Some irregular and scattered acini without colloid left in the center in the midst of lymphocytic masses. A few acini with good colloid are in periphery. In the center lymph vessels studded with lymphocytes. Blood vessels very conspicuous. Uterus transplant very large. A great deal of lymphocytic infiltration in uterine mucosa; surrounding lymph vessels filled with lymphocytes. Much lymphocytic infiltration between and around muscle tissue. Lymphocytes penetrate also into epithelium and partly injure it, but directly underneath epithelium there are only a moderate number of these cells found. Grade 2.5.

COMMENT. We see in these examples variations in the syngenesio-reactions from auto-reactions on the one hand to a condition approaching homoio-reactions on the other. In some cases the center of the piece behaves like that of an autotransplant, but subsequently a lymphocytic infiltration occurs, which in the course of time may become quite marked. In other instances there may be a little increase in fibrous tissue in the center and a more prominent lymphocytic reaction and finally, in still more pronounced cases, the increase in fibrous tissue may extend even around the acini; in the latter event the transplant usually approaches gradual destruction. There is again a parallelism in the behavior of cartilage and thyroid; but as in the guinea-pig the lymphocytic reaction around the cartilage is less marked than around the thyroid. The parathyroid also shows corresponding gradations of lymphocytic infiltration, although in this organ the infiltration is frequently not so marked as in the case of the thyroid. There is on the whole a parallelism in reactions against thyroid and uterus; but uterine tissue may occasionally be

more resistant to the action of syngenesio-toxins than is thyroid. Epithelium, mucosa and muscle layer may be preserved but later a lymphocytic infiltration, varying in strength, sets in. It may affect in some cases the tissue surrounding muscle and epithelium more than it affects these latter structures; but they also are invaded by lymphocytes. The ovarian tissue shows corresponding gradations in reactions. If there is a severe reaction against the thyroid, only certain of the more resistant parts of the ovarian tissue are preserved, such as the layer of germ epithelium (which may form a cyst), interstitial tissue and the medullary ducts. If the thyroid shows a typical syngenesio-reaction, all the ovarian structures, including primordial follicles and various kinds of Graafian follicles with ova are well preserved; atretic follicles also are found. Mitotic proliferation occurs in follicles. Corpora lutea, some of which are newly formed, are preserved. But in cases of syngenesio-reaction lymphocytes begin to infiltrate somewhat even the well preserved ovarian transplant. First they collect around vessels and gradually they may invade even the follicles. A parallelism is thus seen to exist in the intensity of reaction against thyroid and that against ovary, although on the whole the ovary resists better the consequences of syngenesio-toxin formation than the thyroid tissue.

In specimen (f) examined thirty days after transplantation, with a grade of 5.25, in which there was therefore a good syngenesio-reaction, striated muscle tissue was found well developed. This was also observed in other cases. Striated muscle tissue may regenerate after transplantation, at least if the toxic action is not pronounced. The conditions under which this regeneration occurs need further investigation.

SERIES II. TRANSPLANTATION FROM CHILDREN TO PARENTS

(1) *18 to 25 days after transplantation.* Twenty-six experiments. In nine of these cases an auto-reaction was reached or approached; in six cases there was a syngenesio-reaction with slight, in two cases with moderate, and in two cases with marked lymphocytic infiltration; in seven cases there was a homoio-reaction. If we include a few experiments in which the examinations occurred twelve to eighteen days after operation the individual grades are as follows: grade 6 in 4 cases; grades 5.75 to 5 in 13 cases; grades 4.75 to 4 in 4 cases;

grades 3.75 to 2.75 in 4 cases; grade 2.50 to 2 in 4 cases; grade 1 in 1 case. The average grade in these 30 cases is 4.4.

(2) *26 to 50 days after transplantation.* Twelve experiments. In five of these cases an auto-reaction was reached or approached; in one case there was a syngenesio-reaction with slight, in two cases with moderate, and in two cases with marked lymphocytic infiltration; in two cases there was a homoio-reaction. The grades are as follows: grade 6 in 4 cases; grades 5.7 to 5 in 2 cases; grades 4.75 to 4 in 4 cases; grades 2.75 to 2 in 2 cases. The average grade is 4.7. The average grade of the whole group of syngenesiotransplantations (children to parents) is 4.5. This average is not quite so good as the average of the brother to brother transplantations, but nearly approaches it; it is, on the other hand, much better than the average of the homoiotransplantations.

If we compare the reactions in the two types of syngenesiotransplantations between eighteen and twenty-five days, we find in the brother to brother transplantation in nineteen cases an auto-reaction or a slight lymphocytic infiltration, while in five cases the reaction was more severe. In the series of transplantation from children to parents, in nineteen instances we find an auto-reaction or slight lymphocytic infiltration, while a more severe reaction occurred in nineteen other cases; therefore in 50 per cent of the cases the reaction was more severe; while in the brother to brother transplantation the more severe reaction was obtained in only 21 per cent of cases. Between twelve and seventeen days, the relative proportions are similar, namely, auto-reaction or slight lymphocytic reaction in 73 per cent of the brother to brother and in 50 per cent of the children to parent transplantations. The more severe reactions were obtained in 23 per cent of the brother to brother and in 50 per cent in the children to parent series.

During the same period of time the reactions were all severe in homoiotransplantation; the thyroids were either completely destroyed or were on the way to destruction, both connective tissue increase and lymphocytic infiltration being very pronounced. In the syngenesiotransplantation on the other hand, the highest degree of destruction of thyroid and of increase in connective tissue was not usually reached even in the very unfavorable cases. In thirteen cases of this series transplantations were made of the organs of one child to father as well as to mother or of the organs of two brothers

to father or to mother. In seven cases the results in such pairs were the same or similar and in six they were different.

We may cite some examples in order to bring out certain facts relative to the behavior of various organs and tissues after transplantation. (a) *18 days*. Organs of child to mother. Both thyroid transplants well preserved. Acini lying close together, forming a ring. Epithelium low to medium; solid, retracted colloid. In one transplant the thyroid has the shape of an ellipsoid with normal host connective tissue and blood pigment cells in the center, such as we find in autotransplants, while in the other piece the thyroid has a round or oval shape, owing to an increased amount of fibrous tissue in the center, with which are mixed blood pigment cells. In the second transplant there has probably been more hemorrhage, with resulting increase in connective tissue and blood pigment cells. Penetrating the connective tissue are strands of fibroblasts and in addition large blood vessels and lymph vessels, filled with lymphocytes, enter the interior of this central fibrous portion of the thyroid. At the inner margin of the ring of thyroid acini and around parathyroid there is a partly diffuse and partly localized collection of lymphocytes; the lymph vessels are well filled with lymphocytes. In other places lymphocytes are lacking. Only in certain places is there a dense and usually localized lymphocytic infiltration. Some lymphocytes penetrate between the acini. Other parts of the thyroid behave like autotransplants. A few, but on the whole not many, lymphocytes invade also the parathyroid. In both transplants the parathyroid cells are full of mitoses. The cartilage transplant is normal and well preserved, especially the thin parts of the piece. It is surrounded by areolar and fat tissue and in places there is some connective tissue increase. There is no distinct lymphocytic infiltration. Grade 5.

(b) *20 days*. Organs of child to father. Of the uterus transplant only remnants are visible, namely, some well formed cuboidal epithelium surrounding the lumen; also serous coat. Intense lymphocytic infiltration in fat and connective tissue; lymph vessels studded with lymphocytes. Ovary: germ epithelium forms a cyst; interstitial tissue and medullary epithelial ducts preserved. Lymphocytic infiltration near germ epithelium cyst; lymph vessels studded with lymphocytes; otherwise in ovary moderate amount of lymphocytic infiltration. Grade 2.75.

(c) 21 days. Organs of child to mother. Good thyroid ring; solid, good colloid. Medium or low epithelium. In center some fibrous tissue and blood pigment with large vessels, also lymph vessels filled with lymphocytes; very moderate lymphocytic infiltration around vessels. Ovary very well preserved; large follicles and many mitoses in granulosa cells. Good theca interna with capillaries around it. Small follicles and primordial follicles with eggs. Some degeneration of granulosa. Corpora lutea and interstitial tissue well formed. Germ epithelium cyst. Good muscle tissue. Tubes preserved. Fat tissue with some lymphocytes. Grade 5.50.

(d) 22 days. Thyroid resembles lymph gland. Mainly from inner margin of center, but also from outside, large masses of lymphocytes enter septa of thyroid through vessels. Only remnants of acini without colloid are left; the remaining acini are either single or occur in parcels. Lymphocytes penetrate not only between but also into acini and destroy them. A somewhat greater part of parathyroid is preserved; in areolar tissue around it, strands of lymphocytes, also around perichondrium, in places, large collection of lymphocytes. Connective tissue is here newly formed and blood pigment cells infiltrate the tissue. Grade 2.25.

(e) 25 days. Organs of child to mother. Thyroid transplant almost like lymph gland. Dense lymphocytic masses crowd lymph vessels. Great parts of thyroid destroyed. A number of acini with colloid still left; other acini without colloid. Lymphocytes overwhelm and destroy acini and connective tissue grows around them. Some remnants of hyaline vessels. Cartilage well preserved. A certain amount of perichondrial cartilage formation at end. Around cartilage, areolar tissue in which there are here and there moderate but distinct lymphocytic infiltration and some connective tissue increase. Cartilage transplant much better preserved than thyroid. Grade 2.5.

(f) 28 days. Organs of child to mother. Thyroid and parathyroid behave like autotransplants. Cartilage surrounded by areolar and fat tissue; no connective tissue or lymphocytic increase. Bone preserved and also bone marrow with capillaries, osteoblasts, osteoclasts, megakaryocytes. Grade 6.

(g) 33 days. From child to mother. Thyroid transplant with structure of autotransplant. Acini well preserved, joined close together. Solid colloid. Little fibrous tissue in center, but here and in

various places in periphery, lymphocytic infiltration. In certain places lymphocytes penetrate also between acini and enter the parathyroid accompanying the vessels; quite marked lymphocytic infiltration but only small parts of thyroid destroyed. Good parathyroid. Cartilage well preserved. At one end around necrotic cartilage perichondrial regeneration. Much fibrous tissue, but still some areolar and fat tissue around cartilage. In areolar and fat tissue a certain amount of lymphocytic infiltration especially around vessels. A slight mantle of lymphocytes around perichondrium; on the whole little lymphocytic infiltration. Grade 4-5.

COMMENT. These cases illustrate some interesting conditions. In the first case (*a*) the amount of connective tissue developing in the center of the two transplanted lobes of the same thyroid varies considerably, owing in all probability to accidental hemorrhage into one lobe, which led to more organization. In this case also numerous mitoses were found in the transplanted parathyroid cells, both transplants acting in this respect alike. Cases (*b*) and (*c*) are of interest because they represent different intensities of reactions against the transplants. In both instances there is a correspondence between the behavior of the host toward the different pieces. In (*b*) the reactions are severe; the uterus transplant is largely destroyed, and correspondingly only the most resistant parts of the ovary are preserved, namely, germ epithelium, medullary ducts and interstitial tissue; there is relatively much lymphocytic infiltration, while in case (*c*) where the thyroid is very well preserved, all parts of the ovary are in an excellent condition. In cases (*d*) and (*e*) the thyroid transplants both show an intense lymphocytic reaction; in one case there is also some connective tissue new formation between the acini; the parathyroid shows likewise a lymphocytic reaction, which is however less pronounced than that against the thyroid. The cartilage transplants, in particular the tissues surrounding the cartilage, show the least intense reaction, but there is in both kinds of transplants an increase in connective tissue and in lymphocytic infiltration. Case (*f*) shows again the correspondence between the reactions in different organs. Thyroid, parathyroid and cartilage behave like autotransplants; correspondingly bone and bone marrow are preserved; instead of being replaced by fibrillar connective tissue, we recognize typical bone marrow constituents, in particular megakaryocytes. The last case shows in a transplant thirty-three days

old, an auto-structure, but secondarily, attracted by syngenesio-toxins which gradually develop, lymphocytes begin to infiltrate the interacinar tissue. The cartilage shows corresponding changes.

SERIES III. TRANSPLANTATION FROM PARENTS TO CHILDREN

In this series fifty-five experiments were carried out; in twenty-six experiments the pieces were taken out between the eighteenth and twenty-fifth day and in twenty-seven experiments between the twenty-sixth and fiftieth day following transplantation; in two cases the examination took place thirteen and sixteen days after transplantation.

(1) *18 to 25 days after transplantation.* Auto-reactions or beginning lymphocytic infiltration in eight cases. Syngenesio-reactions with moderate lymphocytic infiltration in five instances and with considerable lymphocytic infiltration in six. A condition approaching homoio-reactions was found in seven cases. In the case examined at sixteen days, there was beginning lymphocytic reaction and in the case examined after thirteen days a homoio-reaction was obtained. Grade 6 in 1 case; grades 5.75 and 5.50 in 7 cases; grades 5.25 and 5 in 1 case. Grades 4.75 to 4 (incl.) in 6 cases; grades 3.75 to 3 (incl.) in 7 cases; grades 2.75 to 2 (incl.) in 4 cases, and below grade 2 in 2 cases. Average grade 3.9.

(2) *26 to 50 days.* Auto-reactions in three cases. Syngenesio-reactions with slight lymphocytic infiltration in two cases, with moderate lymphocytic infiltration in three cases, with marked lymphocytic infiltration in six cases. Homoio-reaction or a condition approaching it in thirteen cases. Grade 6 in 2 cases; grades 5.75 and 5.50 in 2 cases; grades 5.25 and 5 in 2 cases; grades 4.75 to 4 (incl.) in 5 cases; grades 3.75 to 3 (incl.) in 5 cases; grades 2.75 to 2 (incl.) in 4 cases; grades below 2 in 7 cases. Average grade 3.2. Total average grade in this series 3.6.

In fourteen cases we compared, in the manner described in the other series, the results obtained in pairs or triplets (transplantation from one parent to two or three children or from both parents to one child). In five cases the same or similar results were obtained while in nine cases the results were different.

We may comment on the findings in a few of our experiments in this series. (a) In a case examined nineteen days after transplanta-

tion, no trace of the thyroid transplant is found on microscopic examination. Uterine epithelium and muscle tissue are preserved, surrounded by dense fibrous tissue. There is fairly marked lymphocytic infiltration, especially in the muscle tissue. The fibrous tissue underneath the epithelium protects the latter, to some extent, from the invasion of the lymphocytes, although some lymphocytes are able to migrate through this layer. It is of interest that in this specimen a certain parallelism exists in the behavior of thyroid and uterus transplants; against both the reaction on the part of the host is marked. Of interest furthermore is the protective influence exerted by the layer of fibrous tissue underneath the epithelium. Grade 2.

(b) The findings were quite different in another case in which the transplants were likewise examined after nineteen days. Here thyroid as well as parathyroid are well preserved, and very large. The thyroid shows auto-structure. Lymphocytic infiltration is very slight. Correspondingly, Fallopian tubes and uterine tissue are also very well preserved, epithelium as well as muscle tissue. In the ovary transplant, well preserved follicles of various kinds, atretic follicles, interstitial gland and a germ epithelium cyst are found. Only a few small collections of lymphocytes observed in the ovarian stroma. Here again correspondence between the behavior of various transplants in the same host is noted. Grade 5.5.

(c) In a case examined after twenty-one days, we had transplanted two lobes of thyroid from the father to the same child. It is of interest that here the reaction against both lobes is the same. In both transplants there is much fibrous and loose connective tissue with fat in the center. Well developed rings of acini are surrounded on the inside and outside by considerable masses of lymphocytes, which penetrate a little into the parenchyma of thyroid and parathyroid. Some acini are surrounded by fibrous tissue. Lymph vessels are crowded with lymphocytes. Grade 3.5.

(d) In a specimen examined after twenty-six days, thyroid, parathyroid and cartilage transplants show the character of typical auto-transplants; of special notice here is the finding of mitoses in epithelial cells of the parathyroid transplant. We have made similar observations in other instances. Grade 6.

(e) In another case in which cartilage and uterus were transplanted from the same mother as in (d) to another child, the areolar and fat tissue around cartilage show much fibrous thickening and

lymphocytic infiltration. In the uterus transplant there is likewise mainly fibrous tissue with very large masses of lymphocytes. A great difference is observed in the behavior of the two children toward organs of the same mother; but the different organs transplanted into the same individual show a corresponding reaction. Grade 1.5.

(f) In three rats examined fifty days after transplantation, the thyroid transplants exhibit a typical auto-structure; especially the center of the transplant shows these characteristics very distinctly. But soon lymphocytes begin to fill the lymph vessels, entering the center of the transplants and from here penetrating into the thyroid ring and destroying parts of the acini; other portions of the thyroid ring, which vary in size in different pieces, are still preserved. Some invasion by lymphocytes takes place from the outside, but this is of much less significance. The parathyroid is also somewhat infiltrated with these cells. The cartilage transplants show some fibrous thickening and lymphocytic infiltration. These cases illustrate the late attack, by masses of lymphocytes, on originally well preserved transplants, the center of the transplants serving as a base from which the invasion chiefly takes place. Grades 4.25, 4.25, 4.

SERIES IV. TRANSPLANTATION FROM GRANDCHILDREN TO GRANDPARENTS

Fourteen experiments were made in this series. Several pieces from various organs were transplanted in each case. Examination took place twenty-five to twenty-nine days following transplantation. Auto-reaction was obtained in three cases; syngenesio-reaction with slight lymphocytic infiltration in five cases, with moderate or more marked lymphocytic infiltration in three cases; conditions corresponding to or approaching homoio-reaction in three cases. Grade 6 in 3 cases; grades 5.75 and 5.5 in 4 cases; grade 5 in 1 case; grades 4.75 to 4 in 3 cases; grades 2.75 and 2.5 in 2 cases; and grade 1 in 1 case. Average grade 4.66.

The average grade in this series is very similar to that obtained in Series II (transplantation from children to parents); here the average grade was 4.5. In transplanting pieces of tissue from the same grandchild to grandfather as well as to grandmother, the reactions may be either the same or different in the two grandparents. If we

transplant pieces from two grandchildren into both grandparents, the reactions against the pieces may be the same in the grandfather as in the grandmother or one grandparent may react more favorably to the transplants from one grandchild and the other grandparent more favorably to the transplants from the other grandchild. We find therefore here the same variations which we found in pair-transplantations in the preceding series.

(a) In one experiment in which the examination occurred twenty-five days after transplantation, the organization of the center of the thyroid transplant is not yet complete; probably hemorrhage has taken place and delayed organization. Fibroblasts and vessels grow into the center; but they also form fibrillar connective tissue around the acini and separate them. Marked lymphocytic infiltration is associated with the connective tissue activity. Lymphocytes migrate into many acini and overwhelm them gradually; however, there are still many areas of acini well preserved. In this case there is at this late period still a formation of fibrillar connective tissue around acini. Of interest also is the good preservation of cartilage, bone and bone marrow; in addition, transplanted muscle is found consisting of muscle spindles with chains of nuclei. In the areolar and fat tissue, around cartilage and bone and between muscle fibers there is marked lymphocytic infiltration and some formation of fibrous tissue. Again we find correspondence in the behavior of the different transplants. Grade 4.

(b) We may cite also a second case, examined after twenty-nine days, in which the thyroid transplant has the typical elliptic shape of an autotransplant with little fibrous tissue in the center. There is increased lymphocytic infiltration at inner edge of the ring of acini; besides the diffuse lymphocytic infiltration in the center, lymphocytes penetrate a little between acini and collect as a larger mass at one pole of the thyroid. Cartilage and bone are well preserved, the former being surrounded by much areolar and fat tissue. Near the bone there is a column of regenerating cartilage cells, becoming necrotic next to the bone. Connective tissue penetrates the bone. Transplanted muscle is attached to the cartilage. Muscle spindles with nuclear chains are noticeable. Of interest in this case is the preservation of the proliferating zone of cartilage near bone and the regeneration of the transplanted striated muscle tissue. Grade 4.75.

SERIES V. TRANSPLANTATION FROM GRANDPARENTS TO GRANDCHILDREN

Twenty-four experiments were made in this series. In two cases the pieces were examined sixteen days after transplantation and a homoio-reaction was obtained. The other cases were examined between the twenty-second and twenty-seventh day and showed an approximate auto-reaction in 4 cases; syngenesio-reaction with slight lymphocytic infiltration in 3 cases, moderate infiltration in 6 and marked infiltration in 3 cases; homoio-reaction in 6 instances. Grade 6 in 1 case; grades 5.75 and 5.50 in 3 cases; grades 5.25 and 5 in 4 cases; grades 4.75 to 4 in 4 cases; grades 3.75 to 3 in 2 cases; grades 2.75 to 2 in 5 cases; grades 1.75 to 1 in 3 cases, and, if we include the two 16 day specimens, in 5 cases. The average grade in this series is 3.5 if we include the 2 cases examined 16 days after operation and 3.7 if we exclude these 2 cases.

In a number of cases we transplanted pieces of tissue from both grandparents into the same child or into several children. The reactions against the pieces from the two grandparents may differ in the same grandchild; in other cases they are about the same. The reactions against the two pieces from the two grandparents may differ in different grandchildren. The character of the donors (grandparents) as well as that of the hosts (grandchildren) influences the result.

The reactions obtained in this series (transplantation from grandparents to grandchildren) are more marked than in the preceding series, in which pieces were transplanted from grandchildren to grandparents. In both cases the average grades obtained are similar to the grades in the corresponding tissues exchanged between parents and children. As in the other series, so we find here also the reactions absolutely less severe in the cartilage than in the thyroid transplant. Lymphocytes are especially observed where there is a connective tissue increase. This combination of connective tissue increase and lymphocytic infiltration is more noticeable in the fat tissue surrounding the cartilage. In other cases both reactions may be independent. This may be observed especially in the thyroid, where lymphocytes not rarely invade secondarily a transplant which shows auto-structure and in which there is therefore no marked increase in connective tissue. In regard to the preservation of various tissues it is of

interest that in one experiment in which the tissues were examined twenty-five days after transplantation, cartilage as well as thyroid is well preserved; in this case bone and striated muscle tissue are likewise in good condition. Grade 5.75.

DISCUSSION

1. If we compare the grades in syngenesiotransplantation in the guinea-pig with those in the rat we find throughout higher grades in the latter animal. The following figures prove this difference very clearly.

SERIES	Guinea-pig grade	Rat grade
I, brother to brother.....	3.6	4.66
II, children to parents.....	2.88	4.5
III, parents to children.....	2.6	3.6
IV, grandchildren to grandparents.....	2.6	4.66
V, grandparents to grandchildren.....	3.0	3.5

If we consider in transplantations in the rat the grades obtained in the earlier period up to the twenty-fifth day we find: in brother to brother transplantations, grade 5.29; children to parents, grade 4.4; parents to children, grade 3.9. The reason for these differences in grades in the guinea-pig and rat series is probably the different degree of inbreeding in the guinea-pig and rat strains used in these experiments. The families in the rat series were obtained almost invariably from strains propagated by the same breeder and the individuals which were mated were usually related to each other, although care was taken that they should not be closely related. In the guinea-pig in many cases individuals obtained from different breeders were mated. We found indication in our previous experiments that the results are influenced by such factors. It will be necessary in further experiments to analyze syngenesiotransplantation in families in which the individuals to be mated are definitely not related to each other.

2. In the guinea-pig as well as in the rat the grades are highest in transplantations from brother to brother. This fact is quite distinct in the guinea-pig and it is also definite in the rat, if we compare the figures for the different periods at which examination occurred. The pieces in the brother to brother transplantations were examined relatively late while the pieces in Series II (children to parents) and

in Series IV and V (exchange between grandparents and grandchildren) were examined at a relatively early date following transplantation. On the average, the grade decreases somewhat at later periods, but this is not invariably the case; it may remain about the same.

In the guinea-pig the difference between grades in Series II, III, IV and V is slight; it is somewhat more marked in the rat. Here the grades in Series II and IV, in which pieces from younger were transplanted to older animals, are better than in the reverse transplantations. Whether this difference in the results is significant or whether it is due to coincidence, is not certain. It is possible that older tissues call forth stronger reactions than younger tissues. On the other hand, there is reason for assuming that if the hosts are animals used soon after birth the reaction will be decreased in intensity; however, animals of this age were not used in these experiments.

3. In both guinea-pig and rat the grades in syngenesiotransplantation are intermediate between those in auto- and homoiotransplantation. This fact is definite in the guinea-pig and it is also distinct in the rat although in the rat transplantations a complicating factor arises consisting in the increase in grades of homoiotransplants in the later period of transplantation. This increase is due partly to a gradual adaptation between host and transplant in the case of cartilage transplantations, but when thyroid is used it is probably due mainly to the fact that we have to deal in the later period with syngenesio- rather than with homoiotransplants. This point is being still further investigated at the present time. However, this intermediate character of the grades is of a statistical character; it represents an average. In individual cases we find all transitions from grades characteristic of autotransplants to those characteristic of typical syngenesio- and in the end of homoiotransplants. In the exchange of tissues between grandparents and grandchildren we find the same variety of grades which we observe in the exchange of tissue between parents and children. It is evident that the individuality differential does not depend upon a single genetic factor. We may represent the individuality differentials in the following manner, which is arbitrarily chosen as far as the actual number of factors involved is concerned. It appears, however, not improbable that the whole genetic composition of an animal constitutes the individuality differential.

Ovum I of Female (mother) A	Spermatozoan I of Male (father) B
1, 2, 3, 4, 5, 6, 7, 8.	a, b, c, d, e, f, g, h.
Individuality differential of tissues of child I: 1, 2, 3, 4, 5, 6, 7, 8; a, b, c, d, e, f, g, h.	
Ovum II of same Female (mother) A	Spermatozoan II of same Male (father) B
1, 2, 9, 12, 14, 16, 7, 8.	a, l, m, n, g, h, r, s.
Individuality differential of tissues of child II: 1, 2, 9, 12, 14, 16, 7, 8; a, l, m, n, g, h, r, s.	

There are thus in the individuality differentials of child I and child II a number of identical factors and a number of factors in one child not represented in the individuality differential of the other. In different cases the number of identical and different factors will vary and accordingly the result of transplantation will differ. The number of strange factors in the individuality differentials of host and transplant may vary between 0 and 16. We may represent in a similar manner the individuality differentials of the tissues of one parent and child.

Mother: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

Child: 1, 3, 5, 7, 9, 11, 13, 15; a, c, e, g, i, l, n, p.

If father and mother had each an entirely different genetic composition, there would be in every transplantation eight strange factors active, whether the transplantation was from parent to child or from child to parent. This would probably lead to a homoio-reaction. Syngenesio-reaction could only be obtained if father and mother had a certain number of factors in common. Under the same conditions the chances for a closer similarity between the individuality differential of host and transplant would be greater in the case of brother to brother than of parent to child or of child to parent transplantation. There should be no essential difference in the reaction observed in the case of parent to child transplantation and child to parent transplantation because in both cases the number of strange factors would be similar.

In this way it may be possible to account in a purely tentative manner for some of the facts in transplantation of tissues. However, we must keep in mind the fact that in addition to genetic factors which are of a specific character, there are other factors which can modify the growth of the tissues and which are of a more general nature. Thus age and perhaps pregnancy also may influence the result of transplantation in other ways than by their effect on genetic factors. Such secondary factors may to some extent alter the average grade of the various kinds of transplants.

4. If we compare the behavior of different organs in syngenesio-transplantations we find a correspondence in accordance with the identity of the relation between individuality differentials of host and transplant in the same experiment. But here again other factors of a secondary character come into play. While the individuality differentials in transplanted thyroid and cartilage are identical in cases of transplantations of these tissues from one animal to the same host the intensity of the lymphocytic reactions called forth by these two tissues differs considerably. This is apparently due to the less active metabolism of the cartilage and surrounding fat tissue and to the less active discharge of the chemical substances which characterize the individuality differential. In regard to the other differences in the reaction against cartilage and thyroid, we refer to our previous papers. The same correspondence is found in the case of bone marrow, uterus and ovary. On the whole, ovary is invaded to a less extent by lymphocytes than thyroid, but the lymphocytic reaction provides also in the case of the ovary an approximate quantitative measure of the relation between individuality differentials of host and transplant. The greater the divergence of differentials, in particular the greater the number of strange factors carried into the host with the transplant, the more severe the reaction becomes. If a certain intensity in toxic action is reached, then the more sensitive constituents of the ovary suffer first; they are destroyed. Thus no well developed corpora lutea may be found, and even ova and follicles suffer. The peritoneal endothelium which tends to form a cyst, the interstitial tissue and the medullary endothelial ducts are longest preserved. Bone marrow is preserved only in auto- and good syngenesiotransplantation; otherwise it is destroyed and replaced by fibrillar or myxoid connective tissue. Of interest is also the finding of well developed striated muscle tissue, when there is a similarity in individuality differentials, as late as approximately a month or longer after transplantation; at this time striated muscle is not found in cases of homoio-reaction. If favorable syngenesio-reactions are obtained in general in these transplants we may observe active mitotic proliferation in the parathyroid cells, while this is lacking under unfavorable conditions. Thus all organs are affected in a similar manner by the degree of relationship of individuality differentials of host and transplant. The lymphocytic reaction presents the finest quantitative determination of these

EXPERIMENTAL GANGRENE PRODUCED BY DIETARY MEANS *

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In the course of a nutrition investigation ¹ in which a large series of albino rats was used, an interesting pathologic condition of the tail of some of the animals was observed. Three groups were studied: a control group on a diet satisfactory for growth; a group stunted by feeding a food low in lysine ² (Ration I, Table 1); and a third group stunted at the same level of body weight by underfeeding with a qualitatively adequate diet (Ration II, Table 1). Of the twenty-one animals on the last or limited calorie dietary régime, twelve developed lesions at the extremities of their tails, which, although varying in the length involved, bore close resemblance to each other and were beyond question of the same nature and due to similar causes.

This tissue abnormality was first noted in rats which were 82 days old † and which had then been on the diet in question for a period of thirty-eight days; it seemed to be a type of necrosis. It is unlikely that infection played a rôle in this lesion since the rats whose tails became involved were not segregated from the other rats used in the experiment. Of the twenty-one rats on a low calorie intake, more than half developed the lesion while not one of the remaining forty-four rats showed any tail necrosis, although the latter were in as close contact with the first group as the individual rats of the first group were with each other. Stunting alone does not explain the phenomenon since the rats which were kept at constant weight by being fed the food deficient in lysine did not develop any demonstrable changes in their tails.

In order to test the possibility of reproducing the above phenomenon as well as to obtain fresh material for histologic study, the experiment was repeated using fifty male rats which were 29 days old. Half of the animals were given Ration III (Table 1), similar to

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† At this age, a normal rat of the sex here used is almost half grown, having passed about one-eleventh of its total life span.

Ration II except that a different preparation of casein was used. Since underfeeding with this diet adds the possibility of protein starvation to energy insufficiency as a possible etiologic factor in the production of the tissue injury, the rest of the rats were given Ration IV, so calculated that while the total energy value of the food given was insufficient for growth, the actual protein intake was the same as that voluntarily eaten by a growing rat of the same weight when Ration III was fed. For example, a rat of 50 gm. body weight growing at the normal rate eats daily about 5 gm. of Ration II and thus ingests 0.9 gm. protein. When a rat is stunted by feeding only 3 gm. of Ration II, it receives only 0.5 gm. protein per day, an amount which, under the conditions of the experiment, may result in protein hunger. By increasing the content of protein to 30 per cent as in Ration IV, a 50 gm. rat given 3 gm. daily receives too small an energy allowance to grow but does obtain 0.9 gm. protein which would be sufficient for normal growth if all other dietary requirements were satisfied. By using restricted quantities of Ration IV, therefore, we are dealing with an uncomplicated energy deficiency.

TABLE I

	Ration I per cent	Ration II per cent	Ration III per cent	Ration IV per cent
Gliadin.....	18
Casein.....	..	18	17†	30†
Starch.....	51	51	52	39
Lard.....	22	22	22	22
Cod liver oil.....	5	5	5	5
Salt mixture.....	4	4*	4*	4*

* Salt mixture of T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, 1919, xxxvii, 557.

† Casein washed with isoelectric water, dried and reground. Analysis showed 13.4 per cent nitrogen, 1.38 per cent ash, 3.04 per cent moisture.

The rats were kept in individual cages with free access to water. Vitamin B was supplied in the form of yeast powder of which each animal received 0.15 gm. every second day. The rats were weighed every other day and a sufficient amount of food given to each to maintain a body weight of approximately 50 gm. Of the fifty rats, three died in the course of the experiment.

Necrosis was first observed in the extremity of the tail of a rat 49 days old which had received the protein-rich diet (Ration IV) for a period of nineteen days. Subsequently other rats became affected. The lesions manifested themselves in much the same manner as

those of the preliminary experiment. The first definite evidence of necrosis in any rat fed Ration III appeared forty-three days after the beginning of the experiment when the rat was 72 days old. The phenomenon, then, is not of accidental occurrence but may be produced at will.

The initial sign of the lesion was the loss of hair from the tip of the tail followed by swelling and reddening at the tip — apparently a typical inflammatory reaction. Next a darkening of the discoloration appeared and then a blackening of the extreme end. Within three to five days, varying in different rats, the black "dot" increased in size and soon a considerable area appeared shrunken, wrinkled and of dark brownish black color. In at least one case the necrosis involved as much as 13 mm. of tail length. The affected region became progressively more desiccated and the outer surface of the tissue sloughed off in flakes of dry, hard scaly material. At the line of demarcation between the dried, darkened area and the normal tissue, there was a swollen and extremely red zone bounded by a ring of retracted skin beyond which was the necrosis proper. After twelve to fourteen days, the entire blackened material had sloughed away leaving for the tail end a swollen, red, blunt tip which was abnormally thick because of the swelling and also because this new tail end was a portion which had previously been an intermediate segment. The tip was now a disc-shaped surface with a hard, white, fibrous tissue core in the center surrounded by a reddened area.

In the preliminary experiments after a period of fifty days upon a low calorie diet, most of the rats were given access to the same food *ad libitum* and growth at an unusually rapid rate ensued. Those tails which had already developed blackened, dry, necrotic tips were not greatly altered by this change of diet. That area of the tail already involved became progressively more and more dried and the process of exfoliation of the outer layers of the necrotic tissue continued until the entire blackened mass had sloughed away. The redness of the new tail ends gradually decreased and no new areas developed. When an extremely small area of the tail was involved and the process consisted merely of a red, indurated tip, neither a progression nor a regression of the process followed.

After the main experiment had progressed for sixty-eight days, four arbitrary classifications were established in order to facilitate

the study of the process. In Group I were classified all rats with tails of the following descriptions: no visible change; normal appearance with a small white tip at the extremity; slightly coarser texture than normal with slight scaliness of the tip; depilation of tip; slight reddening or swelling of tip. These tails were normal or slightly abnormal.

Group II consisted of those rats whose tails manifested marked scaliness of end, irregularity in outline of end, swelling of the tip with bulbous end, reddening of swollen end, slight constriction near end (usually 2 mm. from end) with bulbous portion beyond and beginning of spotting of tail end — red or pink. These tails were somewhat more advanced.

Group III showed a black dot at end of bulbous portion, deep red or cyanotic spots at end and marked constriction with definite swollen bulb beyond. These tails were definitely abnormal.

Group IV included rats showing evidence of dry gangrene varying from one to three or more millimeters of dry, black, scaly tissue and extremely red bulbous ends with black extremities where varying amounts of necrotic tissue had already sloughed away. These tails showed severe necrosis.

Group I included twenty-two rats of which seventeen had received the diet containing the smaller per cent of protein (Ration III) and five rats which had received the diet containing the larger per cent of protein (Ration IV); Group II included eight rats in all, four on Ration III and four on Ration IV; Group III included six rats, one receiving Ration III and five, Ration IV; Group IV included eight rats, one receiving Ration III and seven, Ration IV. Since the groups showing the more advanced stages of necrosis were made up largely of animals provided with the protein-rich food, it appears that there is a correlation between the amount of protein in the diet and the extent of the necrotic lesion.

The same diets were continued for a further period of sixteen days. During this time the lesions progressed similarly to the advance previously noted and at the end of this period examination resulted in classifying four rats previously listed as Group I as now characteristic of Group II and two rats previously of Group II now as typical of Group III.

The process simulated in every respect a form of dry gangrene. The gradual desiccation and desquamation of the tissue indicated a

process of gradually lessened nutrition and finally a total absence of blood supply. It seemed justifiable to assume, therefore, that the described form of gangrenous necrosis of the caudal extremity can be produced by dietary means. It furthermore appeared that the increase in protein percentage of the food hastened the process rather than retarded it.

At the time of classification of the tail lesions into four groups, sixteen tails were amputated and microscopic sections were made using four rats of each group. In four of the cases where the tails were cut, blood counts were made. The number of red blood cells per cubic millimeter of blood varied between 8,356,000 and 9,696,000 — figures within the normal range for the rat.³

MICROSCOPIC EXAMINATION *

A piece of tail about 4 cm. in length was taken which included the necrotic tip and some of the adjacent normal tissue. It was immediately placed in a 10 per cent formalin solution. The block obtained was cut into four portions and cross-sections from each of the four pieces were made in order to give a more nearly continuous picture of the condition of the tissue, advancing from the apparently normal portions to those markedly affected.

Sections of Group I show the normal structure of the tail in those portions most distant from the site of the lesion. The center consists of the cartilage — a rim of cartilaginous tissue arranged in a circular fashion with the enclosed space composed of a lacework of fine tissue. Just dorsal to the cartilage is the artery and just below the artery, a vein. There are also three more veins, one below the cartilage and one on each lateral aspect. Along with the veins can be distinguished nerve bundles. Four muscle bundles extend longitudinally and are placed symmetrically about the cartilage of the center. Advancing outward toward the site of the lesion, there is one place where the intima of the artery has been lifted up and a homogeneous, pink-staining substance is present between the media and the lifted intima. The lumen of the artery can be traced through to the end of the tail. The veins are also patent. The most distal portion shows numerous capillaries. The cartilage is very well preserved throughout.

* The authors are indebted to Professor R. G. Hussey of the Department of Pathology for his aid in the study of the microscopic sections.

In the sections of Group II a few slides show hemorrhages in the walls of the arteries. The veins are engorged and the capillaries are dilated. The cartilage is less well preserved, some sections showing hollowed out portions where the central tissue has fallen out in the process of preparation of the slides. There also seems to be some increase in the thickness of rim of cartilage.

In Group III the arterial wall seems to be somewhat thickened and the outline of the vessels somewhat irregular. Some arteries are filled with a reddish, homogeneously stained substance. The veins in some sections are collapsed and appear to have somewhat thickened walls. No capillaries can be made out. The cartilage is poorly preserved with a thick rim and the inner network of loose tissue sloughed away.

Sections of Group IV contain arteries with quite thick walls. Some veins are collapsed and have thick walls while others are dilated and filled with a homogeneous pink-staining mass. At the tips of the tails there are many hemorrhagic capillaries. The cartilage is poorly preserved and consists of a thick rim surrounded by fibrous tissue with a hollowed out center.

In all sections, the muscle tissue near the extremity of the tail assumes a pink, homogeneous stain suggesting hyalinization. The nuclei of the muscle tissue are pyknotic. The terminal portions of the tails show the dilatation and engorgement as described.

DISCUSSION AND SUMMARY

The origin of the gangrene here observed offers a field for speculation. In general, the causes of gangrene are tissue injury either chemical or mechanical, heat or cold, failure of the general health, circulatory obstruction, nerve disorder, the nerves involved being the vasomotor or possibly the trophic, or microbic infection.⁴ Thus a variety of processes may bring about a gangrenous necrosis, the underlying factor in all being a diminution in the blood supply to the parts affected. However, the production of gangrene is often aided by more general disturbances of circulation or by decreased vitality of the tissues from other causes, as for example in the case of diabetes where gangrene of the feet is so common.⁵ The tendency of gangrene to manifest an upward spread is also well recognized.⁶ In the animals in which the described lesions occurred, a marked degree

of cachexia was produced and it is highly probable that the gangrenous processes observed were due to a principle similar to that recognized as the frequent cause of venous thrombosis in such cachectic conditions as cancer, chronic nephritis, prolonged suppuration and infantile marasmus. In all these cases it is believed that an enfeebled circulation plays an important etiologic rôle, although other factors such as changes in the intima of vessels may well contribute.⁷

The observations indicate that a method has here been evolved in which a form of gangrenous necrosis can be experimentally produced, the chief causative factor in the mortification apparently being the production and maintenance of a cachectic condition of sufficient severity to produce stunting in the experimental animal. Increase in percentage of protein of the diet appeared to accelerate the process. Since the rats showed no evidence of abnormality other than the excessive activity and the rangy appearance due to skeletal overgrowth frequently observed in animals subjected to similar dietary restriction, it would seem likely that the lesions of the tails were local ones. It was also noteworthy that the necrosis was progressive, extending upward by a repetition of the entire process and that the same series of changes manifested themselves repeatedly. The process extended upward not only from the normal tail end or the stump left after the sloughing of a mummified portion of tail but also from the stump left after traumatic amputation of a portion of tail.

It is unlikely that the rats suffered an appreciable degree of anemia since blood counts obtained from four different animals were well within normal limits.

In those cases where the animals were subsequently given free access to the food, the advance of the process was checked although, of course, the portions of tail manifesting definite anatomic change could not be restored to normal.

The microscopic sections showed the most profound changes in the cartilage of the tail. The reticulated structure present in the center of the cartilage in normal portions was absent in those parts near the section of tail which showed necrosis. The rim of cartilage, thin in normal portions, was much thicker and more darkly stained in the other portions. In those parts markedly involved there was a rim of fibrous tissue surrounding the cartilage. The vessels did not show

extreme changes but suggested thickening of the walls and showed engorgement in some sections and were collapsed in others. The muscle tissue showed hyalinization and pyknotic nuclei.

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PATHOLOGIC CHANGES IN THE NERVES OF THE STOMACH WALL IN CASES OF CHRONIC GASTRIC ULCER *

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Although the literature on chronic gastric ulcer is enormous, only a few reports have been made on changes in the nerve tissue in the wall of the stomach adjoining the ulcer. Perman¹ found the nerves surrounded by increased perineural connective tissue, but never any textural thickening of the endoneurium. He observed a few breaks in the continuity of nerves caused by the ulcer. Inflammation of the perineurium was frequent and obvious degenerative changes within the nerves themselves rare. Similar observations were made by Nikolaysen² in 1921, and in the same year Askanazy³ drew attention to something new, namely, that alterations of the nerves adjoining gastric ulcer are far from being always destructive; on the contrary they seem to possess a marked tendency toward a proliferative, regenerative activity. However, none of this work is based on a systematic study of a consecutive series of sections. I have, therefore, subjected thirty-four specimens of chronic gastric ulcer to a careful histologic examination.

The technic employed was as follows: fixation in 10 per cent formalin, embedding in paraffin, serial sections 5 to 7 microns thick; staining methods: hematoxylin and eosin, Van Gieson-Hansen, Mallory's aniline blue method for collagen, and Masson's three-color stain (acid fuchsin-phosphomolybdic acid-jaune métanil).

In most cases I found the nerve tissue pathologically altered (see Table I). The first thing which attracts attention is the abundance of nerve elements as compared with those in sections from normal stomachs. Both the number of nerve branches present and their size are increased. According to my experience the individual nerve

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TABLE I

Alterations of nerves in gastric ulcer

Preparation number	Site of ulcer	Hyperplasia of nervous elements	Nerves passing into ulcer	Nerves embedded in dense connective tissue	Peri-neuritis	Neuromas		
						Periarterial	Auerbach	Diffuse
1 { a b c d	Lesser curvature; 4 cm. from cardia	-	-	A few	(+)	-	-	-
	" " 6 cm. " "	++	Several	Many	+++	-	-	-
	" " 8 cm. " "	+	Many	Several	+++	-	-	-
	" " 10 cm. " "	++	Several	A few	+++	-	-	-
2 { a b	Pyloric orifice	+++	-	Numerous	+	Multiple; situated in retroserous tissue	Multiple	-
	Lesser curvature; 3 cm. from pylorus	+	A few	-	-	-	-	-
	Lesser curvature; middle	++	-	-	(+)	-	One solitary	-
	Lesser curvature; 4 cm. from cardia	+	Several	Many	+	-	-	-
3	Lesser curvature; middle	-	-	A few	-	-	-	-
4	Lesser curvature; middle	+	-	Several	-	-	-	-
5	Lesser curvature; middle	?	-	-	+	-	-	-
6	Near pyloric orifice	+	-	-	+	-	-	-
7	Lesser curvature	+	-	A few	++	-	-	-
8	Lesser curvature; middle	+++	Many	Numerous	+++	One solitary	Multiple; containing nerve cells	-
9								
10	Near pyloric orifice	-	-	-	(+)	-	-	-
11	Near pyloric orifice	+++	-	-	+	-	-	-
12	Lesser curvature; 7 cm. from cardia	+++	-	Numerous	-	Multiple; situated in retroserous tissue	One solitary	-

13	Lesser curvature; middle	++	Several	—	—	—	—	—	—	—	—	—
14	Lesser curvature; middle	+++	—	Several	++	++	—	—	—	—	—	—
15	Lesser curvature; 5 cm. from cardia	(Diffuse neuroma)	—	Numerous	+	+	—	—	—	—	—	—
16	Lesser curvature; middle	—	—	—	—	—	—	—	—	—	—	—
17	Lesser curvature; middle	—	—	—	—	—	—	—	—	—	—	—
18	Lesser curvature; middle	(+)	Numerous	Numerous	+	+	—	—	—	—	—	—
	"	—	A few	Numerous	(+)	(+)	—	—	—	—	—	—
	"	—	Very few	Numerous	—	—	—	—	—	—	—	—
19	Lesser curvature; middle	+++	—	—	++	++	—	—	—	—	—	—
20	Near pyloric orifice	+	Several	Numerous	++	++	—	—	—	—	—	—
21	Pyloric orifice	—	A few	—	—	—	—	—	—	—	—	—
22	Lesser curvature; near pyloric orifice	+	Several	—	++	++	—	—	—	—	—	—
23	Lesser curvature; 7 cm. from pylorus	+++	Numerous	Several	++	++	—	—	—	—	—	—
24	Near pyloric orifice	+++	—	Several	++	++	—	—	—	—	—	—
25	Lesser curvature; middle	+	A few	Several	++	++	—	—	—	—	—	—
26	Lesser curvature; near pyloric orifice	+++	—	Several	++	++	—	—	—	—	—	—
27	Lesser curvature; middle	+	—	—	++	++	—	—	—	—	—	—
28	Greater curvature	+++	Numerous	A few	(+)	(+)	—	—	—	—	—	—

LEGEND: Moderate Reaction +
 Considerable Reaction ++
 Intense Reaction +++

fibrils are not thicker, that is, a genuine hypertrophy of them does not occur. On the other hand, they do show an extremely strong power of resistance against destructive influences and a marked tendency to regenerative activity. In many of the cases examined, moderately large nerve branches are seen to pass through the granulation tissue at the base of the ulcer to terminate directly in the defect caused by the ulcer. Such nerves, apparently perfectly sound, appear, as it were, to be cut by the ulcer.

The behavior of the nerve cells around the ulcers is remarkable. Previous authors have constantly maintained that nerve cells do not occur in the cicatricial tissue. This is not true. Portions of the myogastric plexus extend through the severed muscle coat into the cicatrix and in certain cases may be found directly beneath the granulation tissue layer. Naturally, such nerve cells are pathologically changed. They are small, elongated and arranged in chains and are not unlike the degenerative cells described by Beneke as occurring in certain ganglion neuromas. The cytoplasm is frequently stained deeply (Van Gieson-Hansen) and is sometimes vacuolated. The nuclei are pyknotic, eccentrically located and rich in chromatin. This description includes all the cells considered to be still functioning as judged by the presence of intact nerve fascicles in the neighboring parts. Still more striking changes occur, however, such as blurred outlines of cells, chromatolysis and karyolysis. In certain cases tissue is found containing proliferating nerve cells (preparations 1, 2, 9, 13, 19 and 25) often combined with coexisting inflammatory changes.

The next group of observations concerns the relation of the nerves to connective tissue. The findings may be divided into three groups — (1) the simple passive incarceration of the nerve in the cicatricial tissue; (2) the increase of the perineural connective tissue caused by inflammation; and (3) the growth of interlacing connective tissue fibers within the substance of the nerve. (1) was found in about 50 per cent of the cases examined; (2) is absent in only three cases (10, 16 and 17); and (3) was found but once. On the whole the increase of perineural connective tissue and inflammation are closely connected. This inflammation is toxic or infectious in origin; it may be acute or chronic, the latter being more frequent in this series. The acute forms present the usual picture of emigration and exudation. The chronic form may be congestive or more frequently infil-

trative or sclerotic; any combination may occur. The chief changes are infiltration with lymphocytes and proliferation of the connective tissue. Since the nerves are resistant to inflammation, the changes may be designated as perineuritis in most cases. Rarely the perineuritis is complicated by neuritis; the picture then is essentially an interstitial inflammation, edema and cellular infiltration of the interfascicular tissue. The cells of Schwann are swollen and the vessels dilated and filled with leucocytes; a true suppuration is rare, but does occur.

Due to pressure as a result of the inflammatory processes, the nerves may either atrophy or proliferate excessively (Pitres, Vaillard and Laignel-Lavastine⁴). Thus it seems that the perineuritic changes constitute a connecting link to some forms of hyperplasia and more marked alterations of the nerve. I have not mentioned the purely parenchymatous types of neuritis, because I could not see them as the methods of staining were suitable for only the more obvious degenerative changes.

The majority of the cells in the perineuritic exudation are lymphocytes; occasionally a few plasma cells are found. As the inflammatory process includes the proliferation of fibroblasts, these cells are often numerous and thus make it difficult to determine how far the nerve cell chains extend into the surrounding granulation tissue. The inflammatory changes of the gastric nerves are best seen in the myogastric plexus and in the branches coming from the muscle coats. The perineuritic infiltration as a rule is confined to the proximity of the nerve, not extending into the surrounding tissue. The muscle coats and cicatricial tissue are not infiltrated with lymphocytes until the more chronic stages have been reached. This fact seems to me to confirm Perman's opinion that the lesion may be a secondary, ascending lymphangitis along the perineural lymphatics.

Undoubtedly the inflammation extends along the nerve branches from the ulcer outward; but does this extension take place continuously or does it jump from one place to another, leaving uninvolved tissue between? Perman believes the latter to be true and refers to finding nerves with unchanged perineurium near the ulcer, and inflamed nerves as far from the lesion as the lesser omentum. However, the starting point of the perineuritis is undoubtedly the base of the ulcer. The inflammation may start from the cut nerves or per-

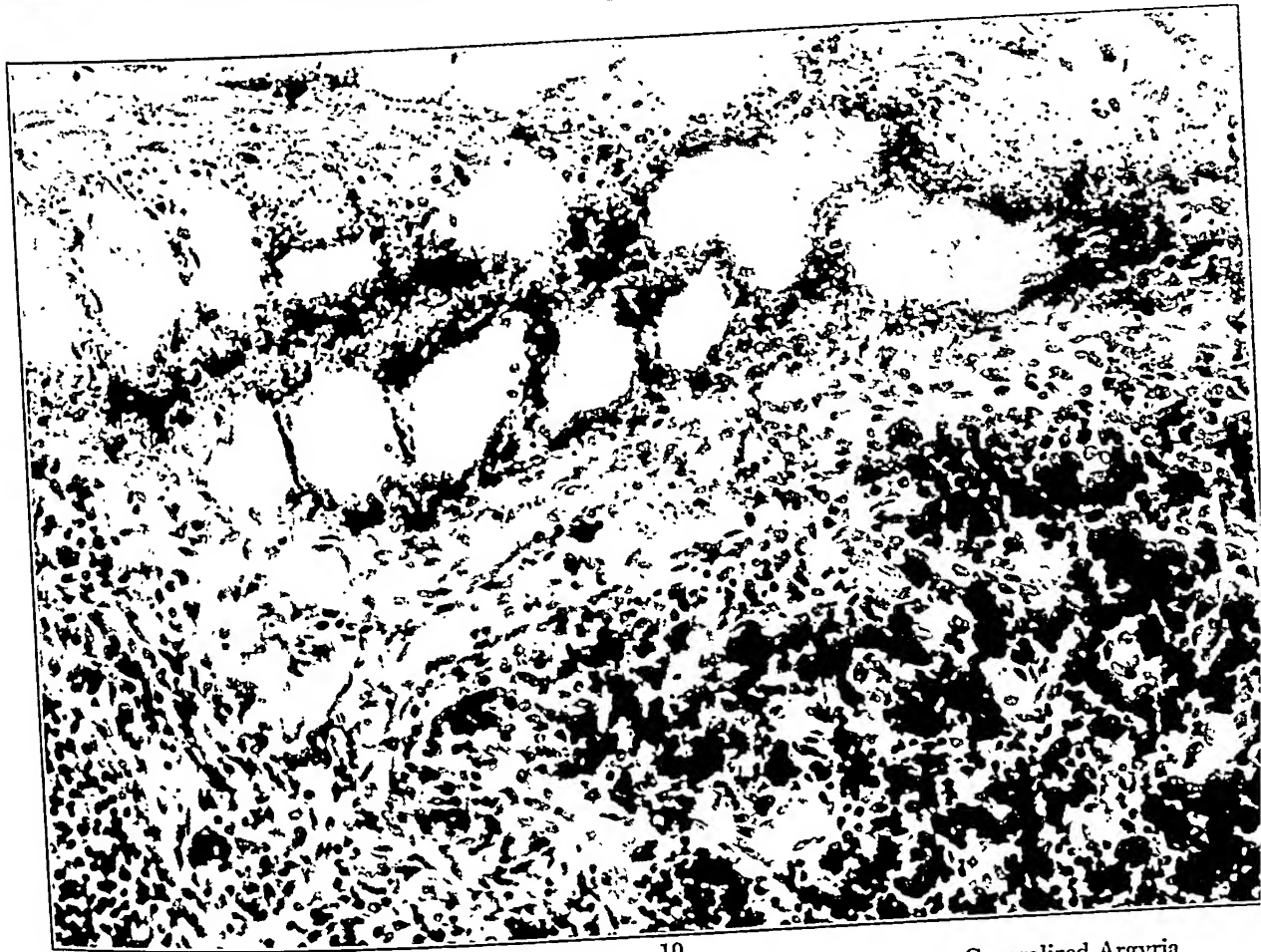
PLATE 171

FIG. 11. Lymph node with rows of silver along the collagen fibers marking out the supporting reticulum of the organ. There are also large masses of silver in phagocytic cells. $\times 500$.

FIG. 12. Sinus of a lymph node showing phagocytic cells containing masses of silver. $\times 500$.



9



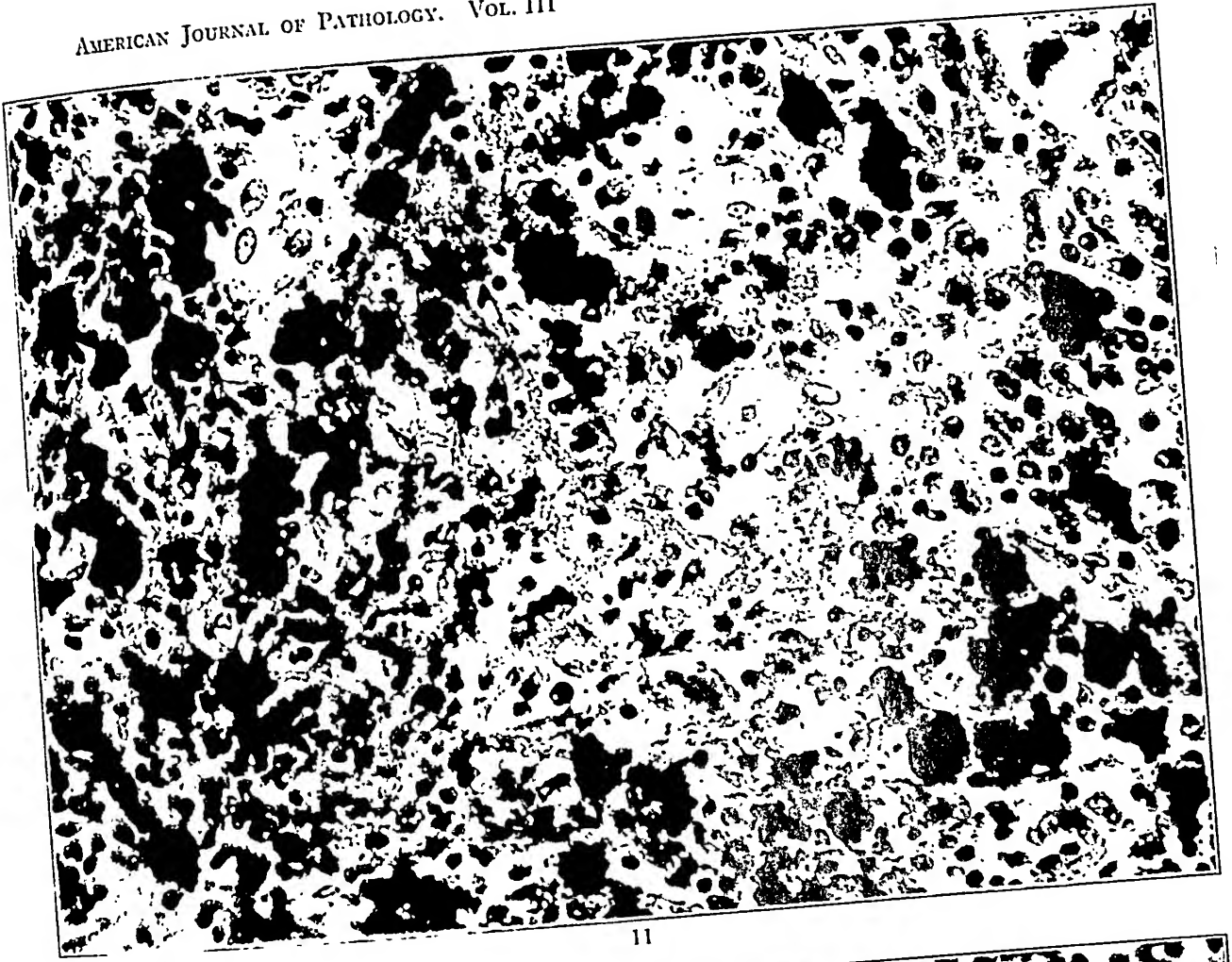
10

Generalized Argyria

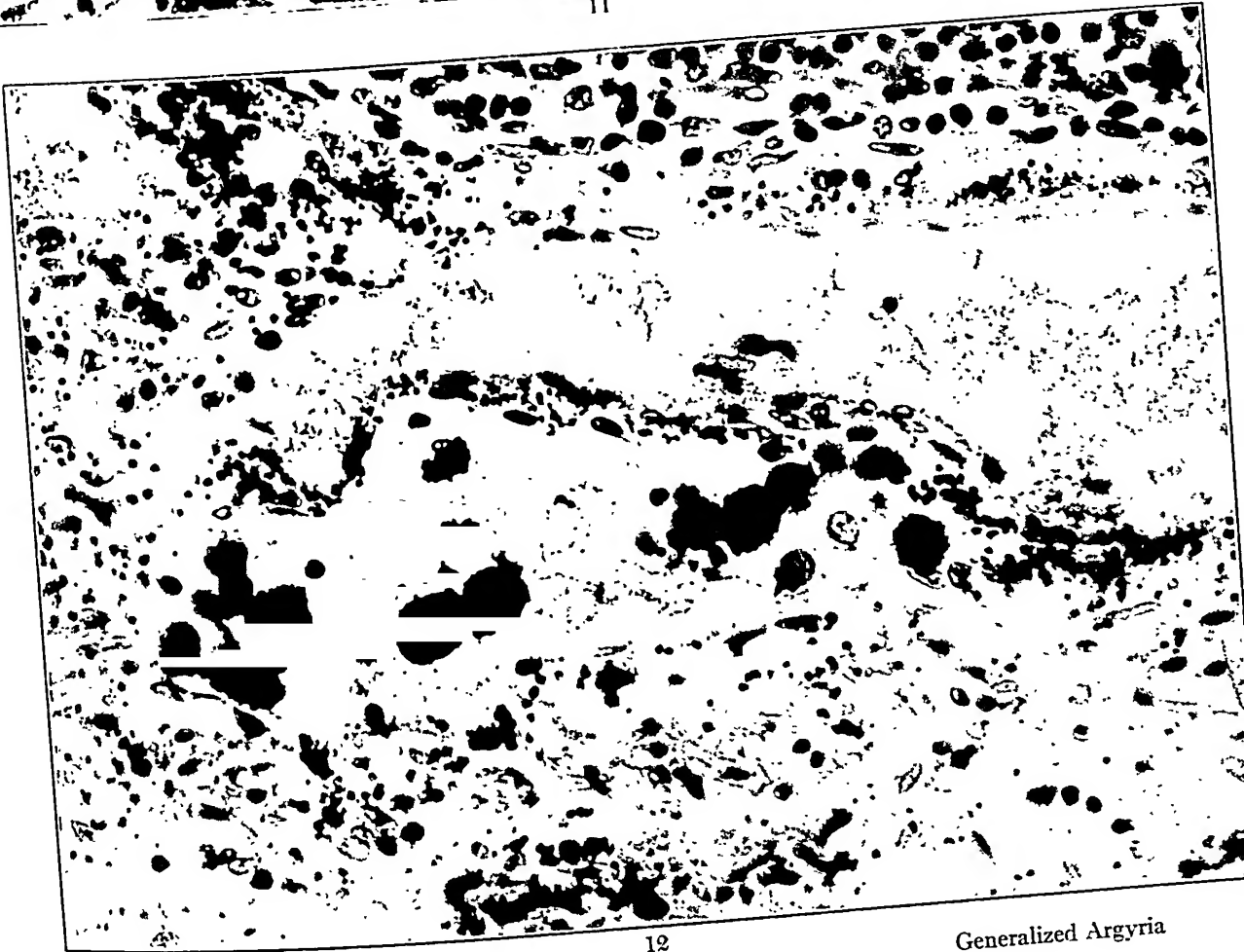
PLATE 172

FIG. 13. Choroid plexus with scattered and massed silver particles in the connective tissue underlying the ependymal epithelium.

FIG. 14. Silver granules distributed along the delicate layer of collagen fibrils which underlie the endothelium of the cerebral capillaries. $\times 750$.



11

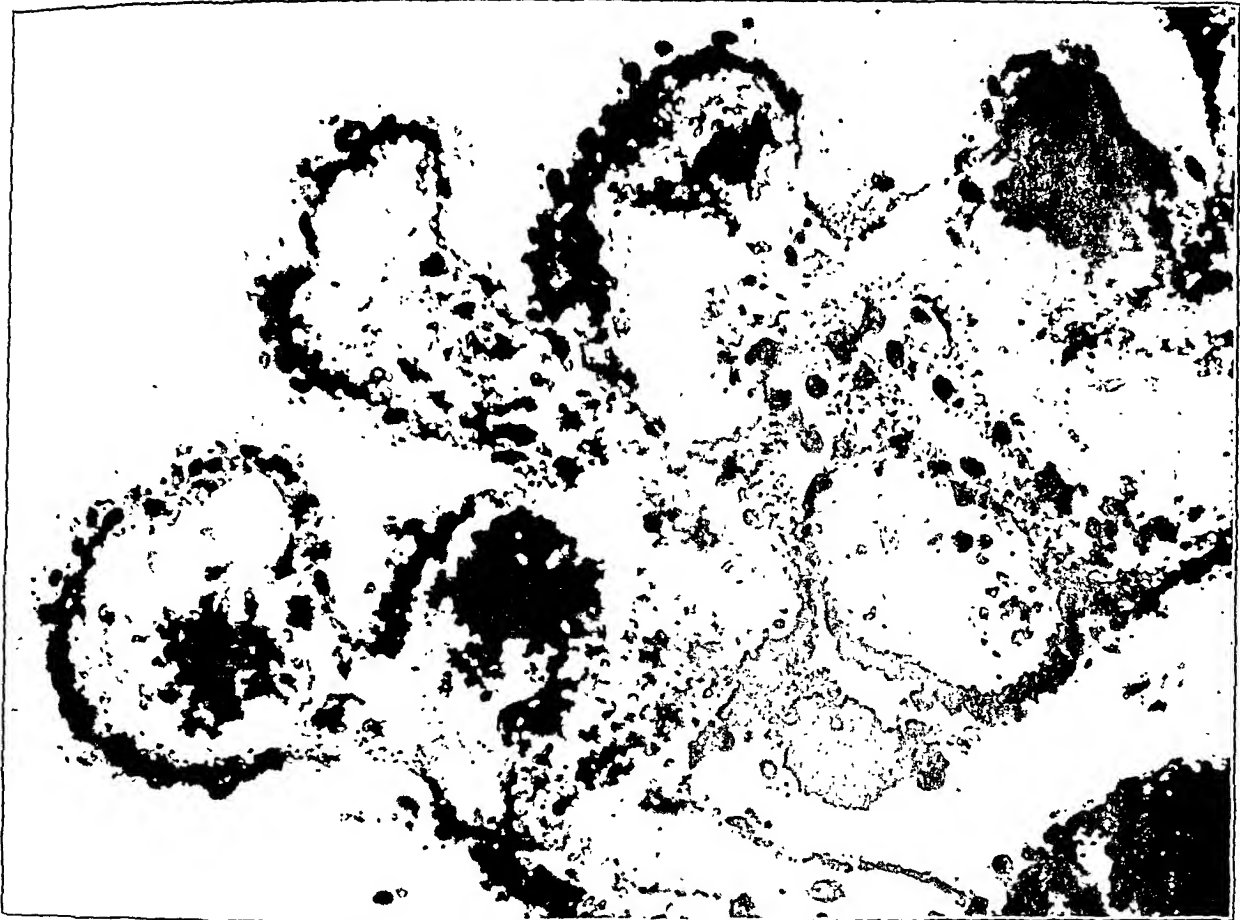


12

Generalized Argyria

PLATE 173

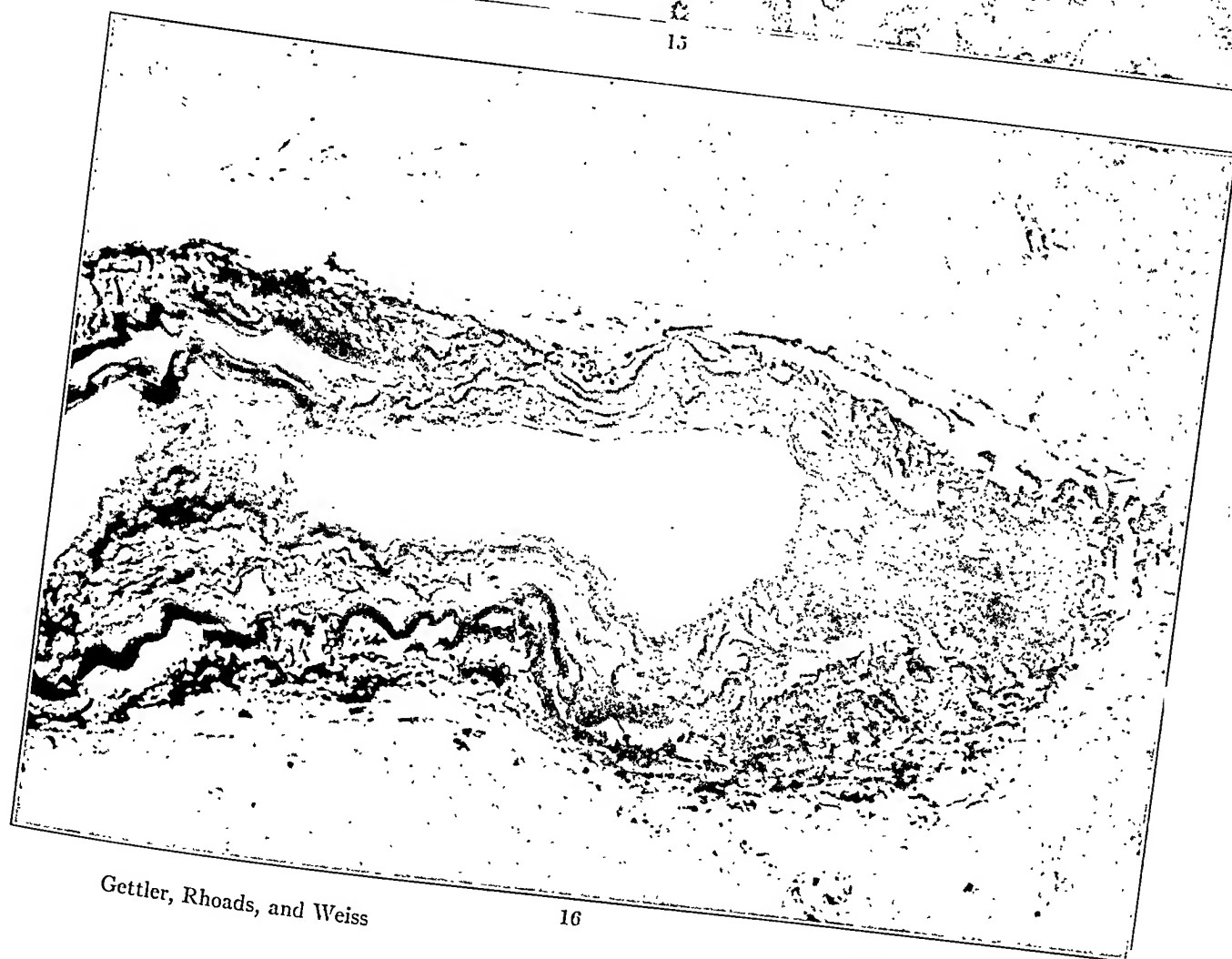
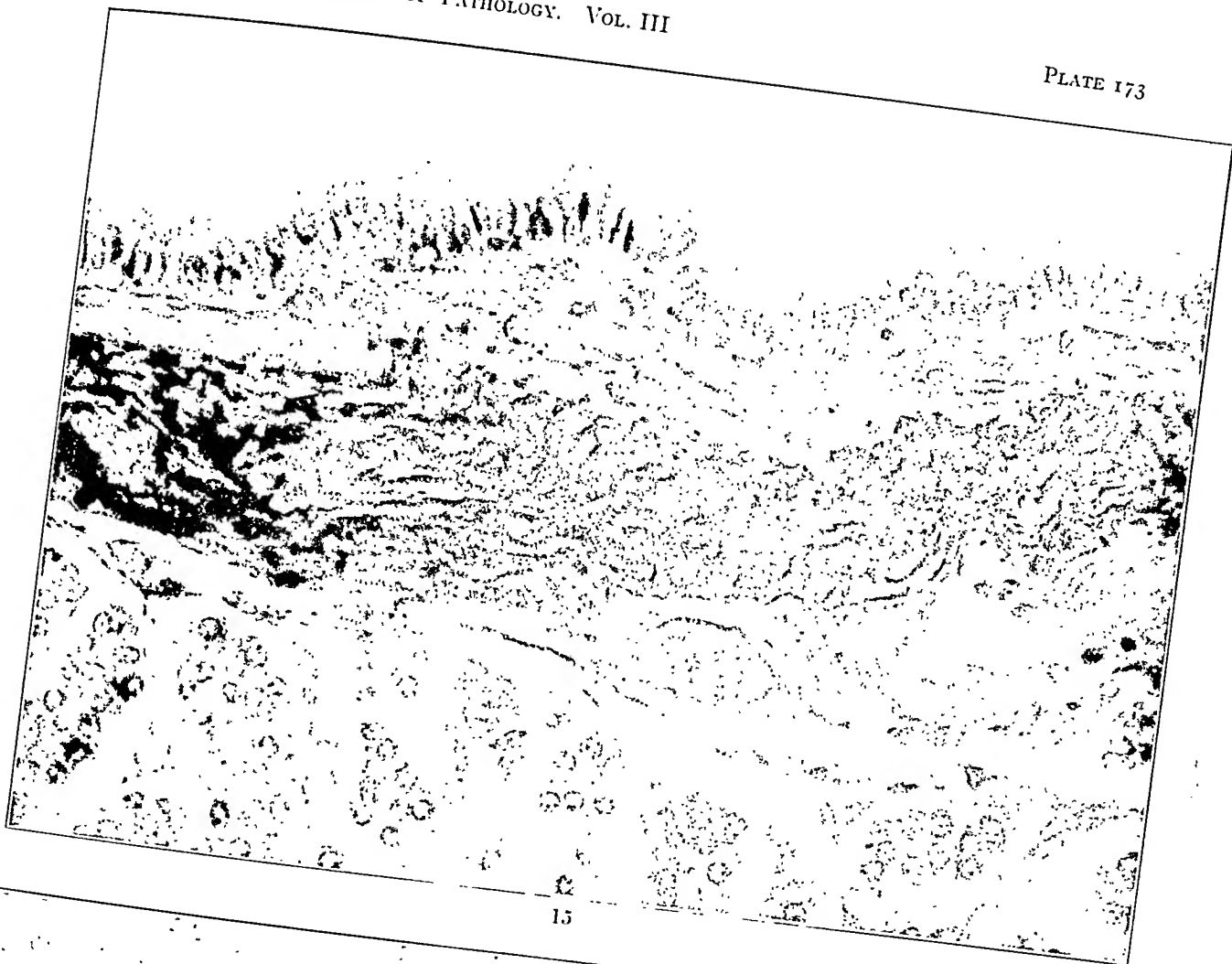
- FIG. 15. Periductular connective tissue of pancreas stained for elastic tissue. This figure compared with Fig. 5 shows the close relation of the metal particles and elastic fibers in this area.
- FIG. 16. An artery of the spleen stained for elastic tissue, which shows that here the silver is deposited in the adventitia outside the outer elastic membrane. $\times 250$.



13



14



Urine: Albumen ++, otherwise negative.

Blood: 8/23/26. White blood cells, 30,000; polymorphonuclears 7 per cent; lymphocytes, 89 per cent; unidentified, 4 per cent. Anisocytosis, poikilocytosis, platelets absent.

8/28/26. Red blood cells, 1,784,000; white blood cells, 9,200; polymorphonuclears, 15 per cent; lymphocytes, 85 per cent.

8/29/26. Red blood cells, 1,790,000; hemoglobin, 50 per cent Sahli.

9/5/26. White blood cells, 10,400; polymorphonuclears, 4 per cent; lymphocytes, 89 per cent; mononuclears, 2 per cent; pathologic cells, 5 per cent; hemoglobin, 50 per cent Talquist. Occasional normoblast and megaloblast, few stippled cells, marked anisocytosis and poikilocytosis, platelets definitely diminished.

9/10/26. Red blood cells, 2,850,000; white blood cells, 11,700; platelets, 30,000 per cmm.; hemoglobin, 55 per cent Talquist.

Stool — Negative for parasitic ova.

Roentgenologic Examination: Heart outline somewhat enlarged, liver and spleen definitely so, otherwise negative. (Fig. 1.)

Clinical Diagnosis: Congenital lues, lymphoid leukemia, thrombocytopenic purpura and rachitis.

NECROPSY REPORT

A necropsy was performed by Dr. Don F. Deeter, resident pathologist, seven hours postmortem. The child is definitely jaundiced, its skin lax and dry, the body somewhat emaciated. Nothing of note is observed previous to opening the abdomen.

Abdomen: Upon opening the peritoneal cavity, a rather large quantity of clear, amber fluid is evacuated. The liver reaches to the right anterior superior iliac spine and the spleen projects several centimeters below the left costal margin. The mesenteric lymph-nodes are slightly enlarged.

Spleen: This weighs 35 gm. and is large, purplish, and shows several small grayish points on its surface. The section-surface is smooth, fairly firm and purplish red; it drips much blood. The follicles are so obscured by the pulp as to be practically invisible.

Liver: The liver is large and weighs 275 gm. Its surface is everywhere studded with small grayish yellow areas averaging 1 to 2 mm. in diameter and projecting slightly above the surface, beneath the capsule. On the section-surface, similar grayish yellow areas are set off from the dull, light brown parenchyma, whose markings are diffuse and obscure. These tubercles are often softened at their centers, as though necrotic. One of them, much larger than the rest, measures approximately 1 cm. in diameter. The gall-bladder is not remarkable.

A CASE OF PRIMARY MESENCHYMAL HEPATOMA: WITH NECROPSY *

NATHAN CHANDLER FOOT, M.D.

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Cincinnati, O.)

This case is of interest to both the clinician and the pathologist, for it presented obscure and misleading symptoms during life and was no less puzzling after a necropsy had been performed.

CLINICAL HISTORY

Present Illness: E. R., a white male baby of four months, was admitted to the pediatric service of the Cincinnati General Hospital on Aug. 23, 1926. The parents said that the child had become feverish and fretful a week prior to admission and that he had been somewhat constipated; no other symptoms were described.

Past History: A full-term, normally delivered baby, the child had been perfectly well since birth; he had never been exposed to contagion.

Family History: The father, mother and one brother were living and well; the mother had borne no other children besides these and there was no history of a miscarriage.

Physical Examination: The patient was a well developed, well nourished child, very restless, but apparently not acutely ill. The temperature was 101.6° F, the respiration 35 and normal, the pulse 140, irregular, but of good quality. Examination of the head, neck and chest failed to elicit anything abnormal; the abdomen protruded beyond the chest; bulging was present in the flanks. No abnormal pulsations were visible, there was no rigidity and but slight tenderness to palpation. A mass could be felt in the right upper quadrant, extending downward to just below the level of the umbilicus and to the left to within 3 cm. of it. It presented a notched, firm border, did not pulsate and the notch was felt just to the right of the umbilicus. Another mass occupied the left upper quadrant, extending downward to the iliac crest and to the right to within 3 cm. of the umbilicus. It had a sharply defined notch in its equally sharp border, felt just to the left of the umbilicus. These two masses were quite distinct, one from the other, the right being firmly fixed, the left one moving with respiration. There was an umbilical hernia and a double inguinal hernia. Aside from bowing of the legs, phimosis and slight icterus, nothing further could be determined.

Course in Hospital: The patient's condition grew steadily worse, despite continued breast feeding from its mother and six transfusions of blood from its father. Diarrhea developed and the temperature rose to 104° F, where it remained for a day or two before the child's death.

Laboratory Findings: A blood culture was negative.

* Received for publication May 18, 1927.

haps more frequently from the numerous spaces between the split fascicles of the perforated muscle coat, where the nerve tissue of the myogastric plexus is always found. When the muscle coat is perforated, a turning point in the history of the ulcer has been reached. The toxic or infectious agents force their way through these spaces in the perforated muscle layers into the nerve tissue and the inflammation continues still farther along the nerves, out through the inner and outer muscle coats to the serosa and on into the omentum. I have followed these changes by means of complete serial sections and am convinced that extension of the inflammatory process is continuous.

The relation of vessels to the ulcer is also of importance. It is a well known fact that small arteries may end freely in the ulcer; the stump of such a vessel is often found thrombosed and surrounded by necrotic tissue. This necrotic zone may be followed through the submucosa and muscularis and represents another portal of entry for inflammation. On the whole, the perineuritic changes are remarkably frequent. In seven of the preparations they are rather slight, and in two entirely lacking. In three of the preparations (4, 9 and 12), I observed a fairly uncommon change in the perineural sheath. The cross-sections of the nerves show a central core surrounded by layers of tissue. This core may consist of regular nerve fibers or may be reduced to a fascicle of Schwann cells. At the periphery there is a gradual transition to unquestionable connective tissue fibrils, concentrically interlaced in a typical manner. Do these layers arise from the perineurium or from the sheaths of Schwann? Staining by Masson's three-color method justifies the view that the structures in question represent transformations of peripheral neuroglia. How this transformation takes place is still uncertain. New investigations have been planned to throw light on this problem.

The last and by far the most interesting of the proliferative changes of the nerves of the stomach are the neuromas. In preparation 15, the following can be found. At a distance of 1 cm. from the organ, the nerve branches from the lesser omentum begin to increase in size and number. They surround the blood vessels, branch out in continuously finer ramifications and are embedded in a meshwork of connective tissue. In the stomach wall there are large masses of nerve tissue, especially in the longitudinal muscle coat which has been split by hundreds of nerves. They vary in size from very large

Gastro-Enteric: There are a few superficial ulcerations or erosions in the gastric mucosa; they average about 1 to 2 mm. in diameter. There is some congestion of the lower ileum and its Peyer's patches are somewhat swollen and prominent.

Other Organs: Nothing remarkable is noted in the case of the kidneys, pancreas, testes, urinary bladder or ureters.

Diagnoses: Multiple gummata of liver, congestion of spleen, ulceration of gastric mucosa, ascites, jaundice, umbilical and double inguinal hernia and rachitis.

MICROSCOPIC REPORT

Technic: Sections of various organs were made in paraffin and stained by routine procedure with Harris' hematoxylin and eosin; in addition, liver and bone marrow sections were impregnated with silver-ammonium carbonate and counterstained by Van Gieson's method; sections of the liver were also stained with Mallory's phosphotungstic acid hematoxylin, carbol-fuchsin and Levaditi's silver method.

General Findings: In the case of the heart, pancreas, testes and suprarenals, nothing of interest is encountered; the kidneys show a well developed tubular nephritis, with marked granular and albuminous degeneration, limited to the convoluted tubules.

Thymus: This shows an unusual atrophy, in that it is composed chiefly of epithelial elements and there is a marked dearth of the normal cortical lymphocytes, with a corresponding prominence of the thymic reticulum cells. The thymic corpuscles are either rudimentary, or show marked degeneration with necrosis at their centers, without any cellular reaction. Many of them have been transformed into thin-walled epithelial vesicles containing a little granular debris.

Spleen: There is pronounced passive congestion that floods the venous sinuses and renders them very prominent, at the same time compressing the splenic corpuscles until they assume insignificant proportions. The sinuses and pulp spaces are thronged with many large, actively phagocytic cells whose cytoplasm is filled with hemosiderin, fragments of erythrocytes and leucocytes, and sometimes entire cells — chiefly polymorphonuclear leucocytes. So numerous are these phagocytes that the picture reminds one of typhoid fever,

but the distribution of the phagocytes, the type of cell they ingest and their general appearance, are quite different. There are masses of cells resembling myeloblasts and myelocytes that, for this reason, indicate myeloid metaplasia.

Lymph Nodes and Lymphoid Tissue: These, too, are atrophic and show a very noticeable reduction in the number of microlymphocytes present; in place of the normal lymphoid follicles, there are aggregations of macrolymphocytes, some of them in mitosis and many of them showing pathologic transformations into large, irregularly staining cells, often with lobulated nuclei and dark cytoplasm. Some of these are very reminiscent of the Dorothy Reed, or Sternberg, giant cells of the Hodgkins' nodes. The central lymph sinuses and the capillaries of the nodules are much dilated and contain large numbers of phagocytes; and fairly large numbers of pale, distorted erythrocytes are found in the vessels and scattered through the lymphoid tissue. Apparently, some process involving the destruction of microlymphocytes, together with a compensatory hyperplasia of their parent cells, has been at work. The lymphoid tissue of the Peyer's patches and intestinal submucosa, on the other hand, is hyperplastic and contains the usual, or a somewhat increased number of microlymphocytes.

Bone Marrow: Sections from the costal bone marrow show some fairly normal fields and, in contrast to these, others are encountered that are strikingly altered. There are sections that are almost entirely pathologic in their composition (Fig. 2) the marrow being invaded by large numbers of large, pale, irregularly outlined phagocytic cells that are, apparently, invading, devouring and replacing the bone marrow. These cells often contain fragments of leucocytes, erythrocytes, or pigment; they are polyhedral rather than of rounded outline, their cytoplasm is vacuolated, their nuclei tend to be vesicular and reniform. They are not only identical, at least in their morphology, with the cells to be described in the case of the liver, but they are imbedded in and intimately associated with a reticulum that is identical in its appearance with that of the tumors. Owing to unevenness of the bone sections, it is impracticable to photograph the silver impregnations.

MICROSCOPIC FINDINGS IN THE LIVER

General: The parenchyma of the organ is compressed and distorted by a growth of new tissue in the periportal areas, or "triads." There is a marked fibrous tissue reaction to the presence of this growth, with consequent interference with the secretion of bile, which is found dammed back in the bile capillaries and the liver cells. Many phagocytes show greenish brown, finely divided particles of pigment in their cytoplasm. The central areas of the lobules are markedly congested and somewhat degenerated.

The New Growth: In the periportal areas are collections of cells quite foreign to this situation (Fig. 3). They are of irregular size and shape, pale, often somewhat vacuolated and usually discrete, arranged either loosely, or in rambling cords, but not in epithelial complexes suggesting alveoli. Sometimes they rim spaces, but even then have very little resemblance to glands. Often they are merely aggregations of cells, each separate from its neighbor and presenting the appearance of the "epithelioid cells" of tuberculosis. In such cases, the aggregations are fairly sharply circumscribed and form spherical, somewhat encapsulated groups; they often show little delimitation and invade the liver parenchyma in their neighborhood by way of the sinusoids. Numbers of leucocytes are often intermingled with these cells, which frequently give the growth the appearance of some sort of granuloma.

The nuclei of the tumor cells are reniform, rounded, or squash- or club-shaped; they are vesicular and pale, with a well defined nuclear membrane and a very poorly defined, or no nucleolus; they are sometimes multiple, two or three presenting in a single cell. Very few mitotic figures are found after most careful and prolonged searching, but as they are in smaller cells it is questionable whether these are tumor cells, or elements of the stroma. A high power photomicrograph of the type cell is reproduced (Fig. 4).

One's first impression, while examining this tumor, is that it has an epithelial origin; for this reason careful observations were made to determine whether or not transitions between the tumor and the liver cords could not be found. Wherever the tumor invades the liver these cords become swollen and degenerated, their cells vacuolated and dissociated, but their nuclei retain their typical round outline and prominent nucleoli. Nucleoli are seldom observed in

the tumor cells. Furthermore, the only cells that resemble those of the tumor with any exactness are found in the walls of the sinusoids, or free in their lumina rather than associated with epithelium, and they are obviously Kupffer cells. Their nuclei are perfectly similar to those of the type cells and their hyaline, pale cytoplasm resembles that of the tumor, which is quite unlike the deeply staining, granular cytoplasm of the liver cords. The liver epithelium, moreover, is often bile-stained and contains large droplets of inspissated bile and encircles dilated bile capillaries; this is not noted in the tumor cells. Pigmented phagocytes lie isolated and included in masses of the tumor, but exactly similar cells are observed in the liver sinusoids; they are quite different in their appearance from the bile-stained epithelium. Where the tumor invades the liver, it does so by way of the sinusoids, rather than replacing liver cords.

As stated, there is a marked fibrous tissue reaction to the presence of the tumor, the stroma (or better, matrix) being chiefly composed of reticulum, although there is a great deal of collagenous connective tissue in the larger, more diffuse areas. This reticulum is so intimately associated with the type cells, not tending to demarcate alveoli, that the question as to whether it is not matrix, rather than stroma, is immediately raised (Fig. 5). In the writer's experience carcinomas very rarely and non-malignant tumors practically never show an invasion of their cell complexes by the stroma; such an invasion always suggesting mesodermal origin of the type cell (Foot and Day¹).

Cell inclusions are frequently observed, although the frank phagocytosis exhibited by the strikingly similar cells in the bone marrow is not found. This might be explained by the firm, closely bound surrounding tissue in the one case and the loosely associated marrow elements in the second; it would be as easy for phagocytic cells to take up myeloid elements as it would be difficult and unusual for them to phagocytose fibers or liver parenchyma.

In order to rule out syphilis and tuberculosis, Levaditi and Ziehl-Neelson preparations were made and examined with quite negative results. Gram and methylene blue stains were equally unenlightening.

Microscopic Diagnoses: Primary mesenchymal hepatoma, probably metastatic to costal bone marrow; chronic passive congestion of liver, with bile stasis and cirrhosis; passive congestion of the

spleen, with myeloid metaplasia; atrophy of thymus and mesenteric lymph nodes.

DISCUSSIONS

This is, then, a case of multiple tumors of the liver with an invasion of the neighboring bone marrow that strongly suggests metastasis, owing to the similarity of the cells in each instance. At first glance one is inclined to diagnose such a case as a granuloma of some sort, tuberculous or luetic. This was the provisional diagnosis at necropsy. But our microscopic examination throws us back upon a diagnosis of neoplasia. Having arrived there, with what type of tumor are we dealing? Primary adenoma of the liver is not uncommon in children, in a rather diffuse and multiple form; but can we consider this an adenoma? Can it, indeed, be considered to be of an epithelial nature? The type cell suggests epithelium, but it also suggests mesodermal origin.

Arguing against an epithelial origin we may point out that:

- (a) There is no tendency for the cells to form glands or definitely coherent alveoli.
- (b) There is great similarity between the cells of the tumor and the phagocytic interlopers in the costal bone marrow.
- (c) There is a tendency for the cells to be multinuclear.
- (d) They are usually discrete and very intimately associated and intermingled with reticulum, which is more typical of reticulo-endothelial, than it is of epithelial structures.
- (e) They resemble Kupffer cells more closely than they do hepatic epithelium.
- (f) They invade the liver tissue by way of the sinusoids, rather than replacing the liver cords.

In favor of an epithelial origin of the tumor the following points may be noted:

- (a) Tumors corresponding to this type of neoplasm are usually epithelial.
- (b) The cells of this tumor resemble epithelium closely enough to be termed "epithelioid," they show some tendency to form cords and a very slight one to line spaces.
- (c) Ducts are occasionally seen in the tumor, but these could be construed as representing surviving bile ducts.

It is quite possible that there is no relationship between the cellular aggregations in the bone marrow and the tumor in the liver; the former are composed of much larger cells, as may be seen in the illustrations, which were taken at the same magnification. The increase in size, however, could be explained on the ground of compression in the dense tumor and lack of this in the loose marrow. Metastasis, in the absence of numerous mitoses, is not as readily assumed as it could be were there many of these; but the tumor has grown readily enough throughout the liver and could, therefore, set up subsidiary growths outside of that organ. That this tumor is malignant is indicated by the poor differentiation of its type cells, by their diffuse and infiltrating growth and the possibility of metastasis to the ribs; that it was clinically malignant is amply proved by the fact that it killed the patient.

The most comprehensive description of these tumors will be found in Ewing's *Neoplastic Diseases*.² He lists primary tumors of the liver under the following heads: (*a*) Solitary adenoma, (*b*) Primary massive liver cell carcinoma, (*c*) Multiple liver cell carcinoma or hepatoma, and (*d*) Carcinomatous cirrhosis; or, multiple adenoma, carcinoma, or hepatoma with cirrhosis. The tumor under discussion might fall into the last category, were we certain of its epithelial nature. Ewing mentions the fact that Geraudel considers some of these to be of mesodermal origin, but dissents from this view on the grounds that the liver "is derived from exactly the same endodermal bud as the bile ducts, and the only part of the parenchyma which is of mesodermal origin is the system of blood vessels."

If our tumors, then, be not of endodermal origin, they are probably derived from the vascular primordium of the liver, or from its appendages which produce the hepatic stroma. For the reasons already given, it seems to the writer that we are dealing with a primary mesodermal tumor of the liver which has grown diffusely, either arising simultaneously in many parts of the organ, or rapidly seeding itself out from the largest nodule discovered at necropsy and possibly metastasizing to the neighboring ribs. Such a diagnosis may seem presumptuous, in the face of tradition, but we have ample evidence that the Kupffer cells may form large aggregations in tuberculosis, and in other conditions. The experimental work on tuberculosis of the liver has shown this to be true (Evans, Bowman, and Winternitz,³ Foot,⁴ Kockel,⁵ and others). Why should these cells

not be capable of undergoing somewhat similar changes under the influence of neoplastic stimulation and become transformed into actively growing tumor masses? This hypothesis will bear consideration; it is only by publishing such cases and inviting free criticism and discussion, that we can arrive at any definite conclusion as to their true nature.

NOTE: Personal correspondence with Dr. Ewing indicates unmistakably that he considers this case to be one of multiple adenoma of the liver. Although he examined the sections from the liver, he did not see those from the marrow, which attracted attention only after the liver sections had been studied and sent on to him.

SUMMARY

A case of multiple tumors, occurring in a boy of four, is described from the clinical and pathologic points of view, the latter being stressed. The tumors simulate multiple granulomata, possibly tuberculous or luetic, but when examined more closely are found to be neoplastic. They are rich in reticulum and are found to invade almost all the periportal tissue of the liver. Similar cells and reticulum are found in the neighboring costal bone marrow, which are probably metastases to that tissue, although this is not proved. The nature of the growth is discussed, and it is concluded that it probably had its origin in the mesodermal primordium of the liver; the possibility of its being of epithelial origin has not been overlooked.

Photomicrographs by Mr. J. B. Homan (Dept. Medical Art, College of Medicine, University of Cincinnati) and the author.

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DESCRIPTION OF PLATES

PLATE 174

FIG. 1. Roentgenogram of the patient, showing enlarged liver, spleen and heart; no metastasis visible in ribs.



PLATE 175

FIG. 2. Photomicrograph of the bone marrow, showing invasion by large numbers of phagocytes, possibly a metastasis. Hematoxylin-eosin. $\times 200$.

FIG. 3. Photomicrograph of the tumor and the liver tissue at its margin. Note the lawless distribution of tumor cells. Hematoxylin-eosin. $\times 200$.

nerve fibers to innumerable minute fibers, and they emerge in the compact tela submucosa. The stomach wall is interlaced in this manner for a space about 3×6 cm.; in an area a little larger than 1 cm. square, I counted 150 medium-sized nerves besides innumerable smaller ones. On the whole, the branches showed no conspicuous anatomic changes. This lesion is called a diffuse neuroma.

In preparation 9 there can be seen along a medium-sized nerve a small neuroma embedded in fibrillar connective tissue. The neuroma macroscopically appears as a spot the size of the head of a pin. Its component parts are arranged in an irregular manner and they penetrate between the remnants of the longitudinal muscle coat. The neuroma contains numerous small polygonal nerve cells which are deeply stained and show definite processes. The nuclei are small, pyknotic, contain ample chromatin and are centrally located. The nerve cells are embedded in fairly dense tissue and are scattered irregularly through the neuroma. In another series from ulcers of the same type, similar observations have been made, as in preparations 2a, 3, 12, 18a, 26 and 28. We are confronted with the occurrence of multiple small neuromas arising in the central portion of the cut nerves of the myogastric plexus. In every case there is a small central cicatricial neuroma and since these contain a special type of nerve cell, I suggest the name Auerbach neuromas.

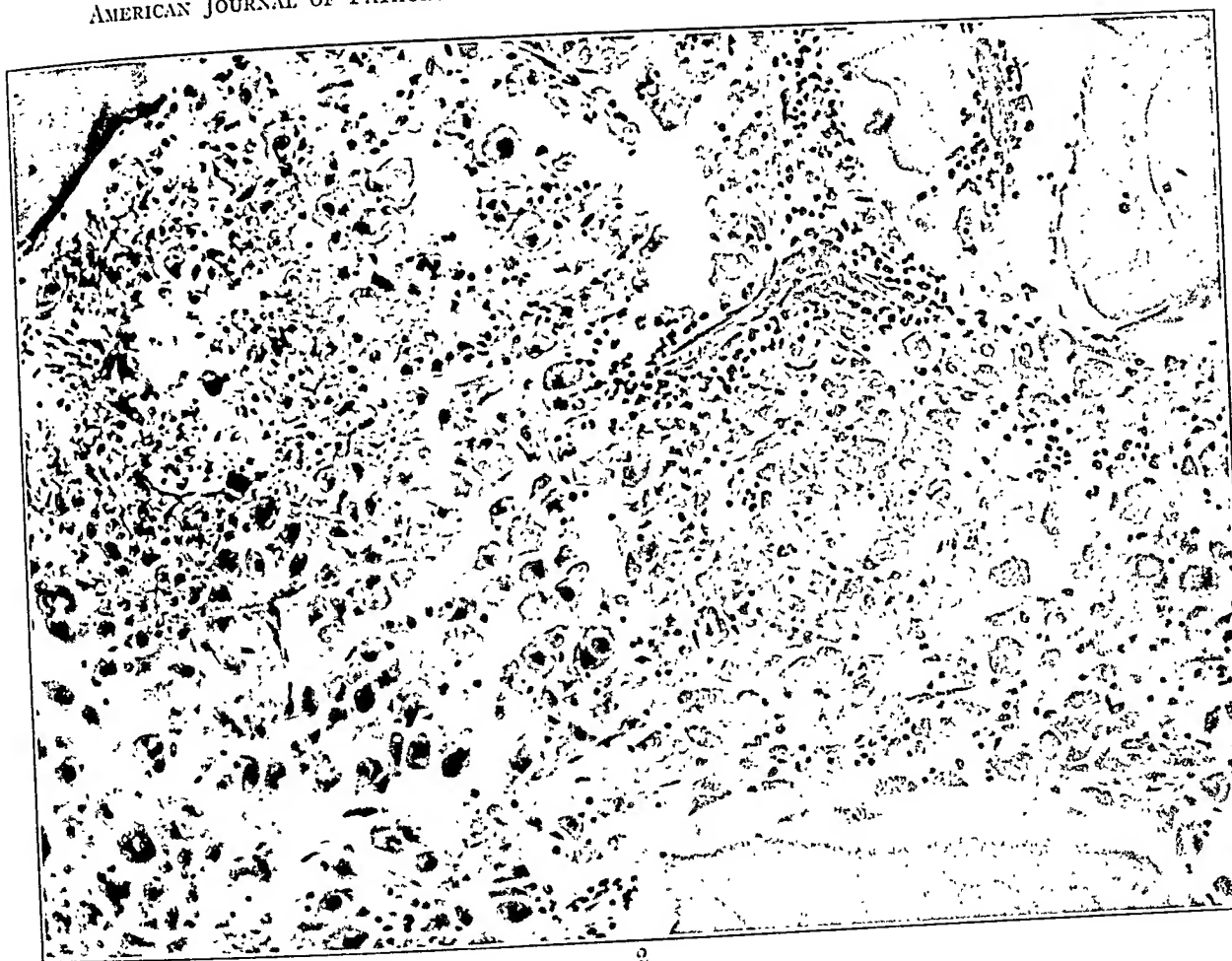
In case 2a, a third type of central cicatrix neuroma occurs. Near the lateral wall of the large ulcer is a conglomeration of arterioles the size of a hazelnut kernel. Surrounding a group of eight small arteries in this mass are nerves running in various directions and embedded in a coarse meshwork of fibrillar connective tissue. Many of the nerve branches are subject to obvious inflammatory changes. Similar conditions have been found in preparations 9, 12 and 14. I have entered them in the table under the heading periarterial neuromas. Thus two fairly well defined types of cicatrix neuromas exist, the periarterial neuroma and the Auerbach neuroma, while the diffuse neuroma occupies a unique position.

I am of the opinion that no genuine tumors have been reported in the cases recorded, neither ganglion neuromas (Beneke⁵), nor "sympathomes" arising from persisting embryonal neuroblasts (Masson⁶). On the whole, my observations confirm Askanazy's theory that by these proliferative changes we are confronted with a type, the cicatrix neuroma (Virchow) akin to the amputation neuroma. A

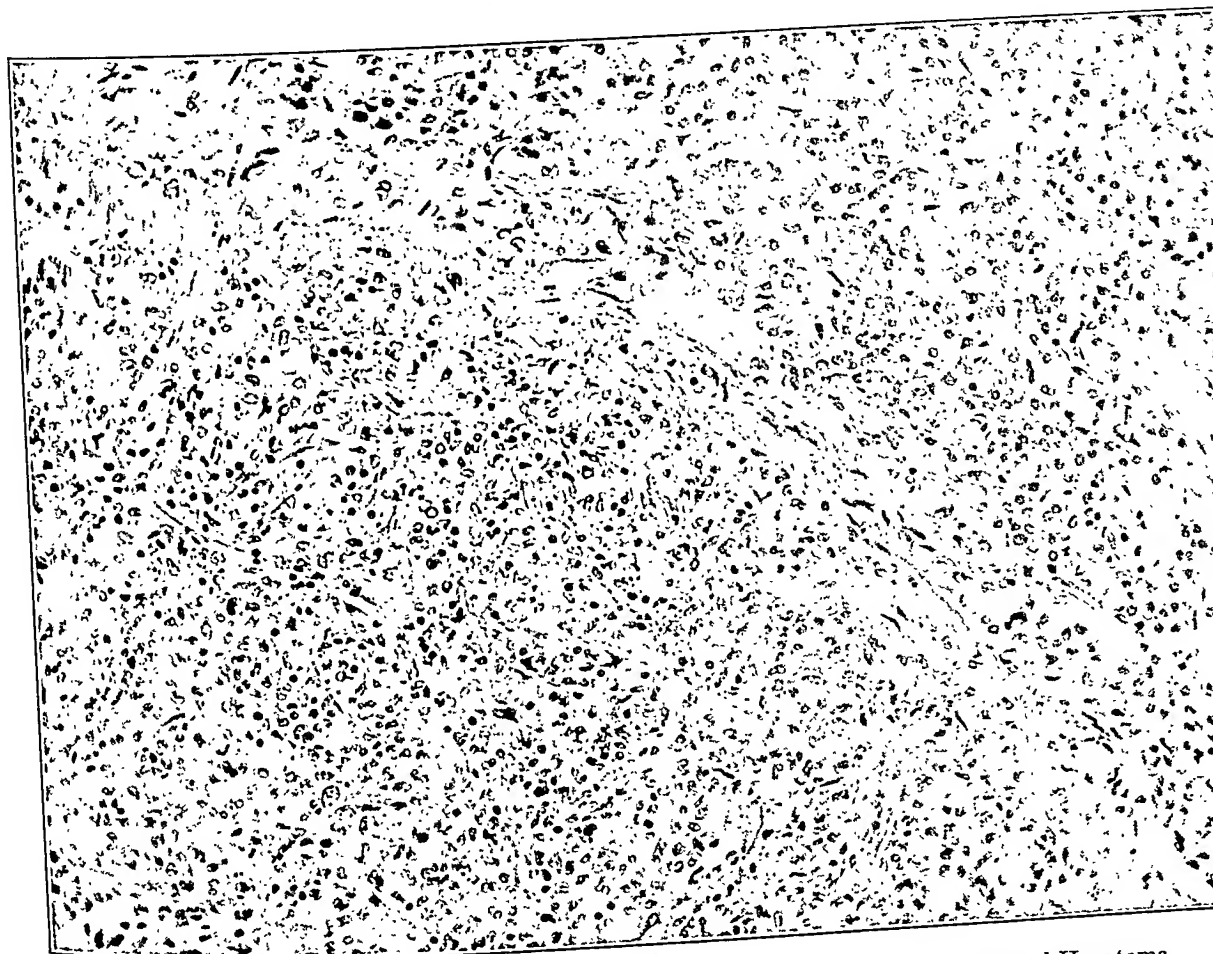
PLATE 176

FIG. 4. Photomicrograph of a field in the tumor, to show the distribution of reticulum and the type cells. Silver-ammonium carbonate-Van Gieson technic. $\times 800$.

FIG. 5. Photomicrograph of a portal area completely replaced by tumor tissue. Note distribution of reticulum and dissimilarity of the tumor and liver cells. Silver-ammonium carbonate-Van Gieson technic. $\times 200$.



2



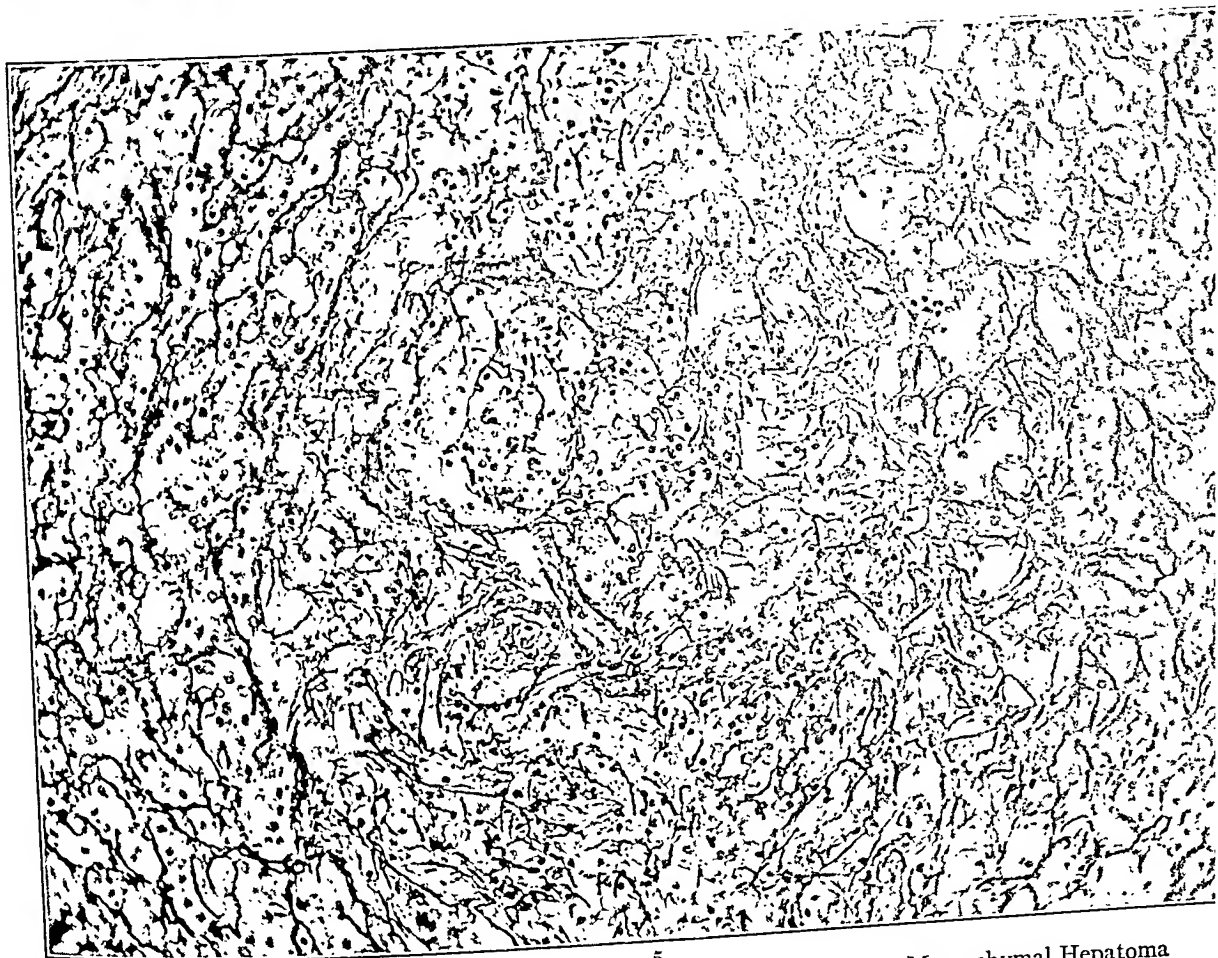
3

Foot

Primary Mesenchymal Hepatoma



4



5

Foot

Primary Mesenchymal Hepatoma

per cent of the experimental animals were found to harbor this parasite. More recently Darling⁴ has been able to transmit this infection to guinea pigs by similar methods, but this does not account for the manner in which the parasite gains entrance to hosts that are strictly herbivorous. In further experiments Theobald Smith⁵ has proved that this organism was not transmissible from parents to their offspring.

Sarcosporidia in the human were first described by Rosenberg⁶ in 1892, who found small round or oval refractile bodies in a cyst in the papillary muscle of the left ventricle in a woman of 40 years, dying of pleuritis and endocarditis. According to Fantham, Stevens and Theobald⁷ there is some doubt about this case being one of true sarcosporidiosis. The first authentic instance of the appearance of this parasite in man was reported by Kartulis⁸ in 1893, who found Miescher's cylinders of various sizes in the muscles and possibly in the liver of a Sudanese who succumbed to multiple abscesses of the liver and abdominal muscles. Baraban and Saint-Remy⁹ found undoubted parasites in the laryngeal muscles of a man who was executed. Darling reports two cases of sarcosporidiosis in man. The first¹⁰ was in a piece of biceps muscle obtained from a negro working in the Canal Zone and suffering from typhoid fever. In a biopsy performed four months later no parasites were seen, so it is possible that they are only transient invaders of the human host. Darling's second instance¹¹ was in an East Indian who died of malaria, and sarcocysts were found in sections of the muscles of the tongue. Cone¹² reports a case of multiple bone cysts from which he recovered bodies resembling the sarcosporidium, as well as yeasts. After feeding rats some of the infected material he found similar bodies in their muscles but it seems possible that all the bodies described by him were yeasts. The last case to which we have found reference is that of Manifold,¹³ published in 1924. In sections of heart muscle three parasitic cysts (A, B, and C) were found, and two others in sections of tissue in the vicinity. Numerous other blocks were examined later without finding additional parasites, so the infection, it was inferred, must have been slight. The lining membrane of the cysts stained well, but no trace of hair-like processes could be seen. The cysts contained the usual sickle-shaped spores, which were evenly distributed and did not appear to be packed into spore chambers. No evidence of a reticulum could be made out. The sur-

SARCOSPORIDIAL INFECTION OF THE MYOCARDIUM IN MAN *

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(From the Laboratory of Pathology, Presbyterian Hospital, New York, N. Y.)

The first instance of sarcosporidial infection was discovered in mice by Miescher¹ in 1843, who described white filaments running parallel with the direction of fibers of voluntary muscles. Since that time sarcosporidiosis has been found very commonly in cattle, horses, sheep and pigs and also in various other mammals, as well as birds and reptiles. Wenyon² gives a complete list of the animals in which this parasite has been found together with the different species, but it is possible that they are all of the same species, taking slightly different forms in the different hosts. Although these protozoa are usually non-pathogenic even in very heavy infections, one form, the *S. Muris*, is lethal to mice and at times may cause fatal epizootics in sheep.

The sarcosporidia are characteristically surrounded by a membrane or capsule, which in the younger cysts, cannot always be demonstrated. In the older ones this becomes quite definite and occasionally appears to be radially striated. It has not yet been decided whether this surrounding membrane develops from the parasite itself, or is formed about it by the adjacent cytoplasm. Growth of the cysts occurs in the periphery. At the same time the central portion may become necrotic. In the older sarcocysts thin trabeculae often run from the capsule through the interior, forming chambers which contain the spores. These spores vary in shape, but usually are kidney- or sickle-shaped and measure 10 to 15 microns in length.

The natural mode of transmission of this parasite is not known, but the infection has been produced in mice by Theobald Smith³ by feeding them infected mouse flesh. The sarcocysts could not be demonstrated in these mice until forty-five days after the infected material had been administered to them. After this time about 63

* Received for publication July 19, 1927.

NECROSPY REPORT

Anatomic Diagnoses: Acute and chronic arteriolitis; chronic nephritis (arteriolar); cardiac hypertrophy (left ventricle); acute endocarditis, mural (rheumatic); lobular pneumonia; hemorrhage into cerebellum; sarcosporidiosis of heart; fibromyoma of uterus; hydrosalpinx; sclerosis of pulmonary venules; fibrous pleural and peritoneal adhesions.

The anatomic diagnoses sufficiently state the findings in the case so that only the heart will be described.

HEART

Gross Description: Weight 360 gm. The heart is enlarged, the enlargement being due chiefly to hypertrophy of the left ventricle which is firmly contracted; the right ventricle and auricle are dilated. There are a few small epicardial hemorrhages on the surface of the right auricle. The myocardium is of the normal red-brown color except on the septal portion of the left ventricle where there are numerous subendocardial hemorrhages of considerable size. The endocardium is smooth and glistening throughout. The valves are thin and delicate except for a small atheromatous plaque in the aortic leaflet of the mitral valve. The coronary arteries show slight atheroma.

Microscopic Examination: Sections from the left ventricle show some hypertrophied muscle fibers, some with vacuoles and some that are fragmented. Throughout the myocardium are a few small areas of fibrosis, and about the blood vessels there is some lymphocytic infiltration. Immediately beneath the endocardium are a number of polygonal cells with rather basophilic cytoplasm and directed more or less vertically to the surface. In several places they are grouped into submiliary nodules which project above the surface and one of these is covered with a fresh deposit of fibrin. The walls of the blood vessels are somewhat thickened.

In sections from the interventricular septum are seen a few scattered parasites lying in individual muscle fibers. These fibers are swollen to about twice their natural size and stain more homogeneously than the other fibers in the sections. However, the striations can be seen faintly and the fibrils are easily distinguishable in the

rounding muscle appeared quite healthy in two of the cysts, but was somewhat hyaline in the third.

The dimensions, in comparison with Darling's case, are given in the following Table: In the last column the measurements of the parasites in our case are appended.

	Manifold's case			Darling's case	Lambert's case
	Parasite A	Parasite B	Parasite C		
<i>Sporozoan:</i>	mm.	mm.	mm.	mm.	mm.
Length057	.045	.034	.084	.082
Width045	.039	.026	.027	.031
<i>Spores:</i>					
Length01092	.01082	.01092	.00425	.0072
Width00156	.00136	.00156	.00175	.002-.0025

Report of Case: R. B., age 32, negress, housewife, married, born British West Indies.

History: Admitted to the hospital Sept. 18, 1926, she died Sept. 19, 1926 (history from sister). Always well until four months before admission when she commenced having headaches, anorexia, frequency of urination and blurring of vision, all of which persisted up to ten days before admission. At that time she became much worse, being forced to bed by transitory blindness, continued severe headaches, pain in back of neck and intermittent pain in kidney region. On the night of admission she became completely disoriented.

Physical Examination: Temperature 99.2° F; Pulse, 120; Respiration, 20; Blood pressure, systolic 270, diastolic 170.

A poorly developed negress, active and argumentative. Pupils negative. Eyegrounds showed white exudate and hemorrhages. Heart not enlarged. Soft systolic apical murmur. Aortic second sound markedly accentuated. Pulse strong. Vessels thickened, not tortuous. Deep reflexes more marked on left than on right.

Laboratory Findings: Hemoglobin, 100 per cent; red blood cells, 7,300,000; white blood cells, 12,200; polymorphonuclears, 78 per cent; lymphocytes, 12 per cent; endothelials, 10 per cent. Urine: Specific gravity, 1014; albumen, heavy trace; no casts. Some white blood cells in clumps. Lumbar puncture: fluid under greatly increased pressure. Cells: lymphocytes, 30; red blood cells, 310; globulin, slightest possible trace. Wassermann: negative. Colloidal gold, 0011221000. Protein 75 mg. per 100 cc. Blood urea .55 gms. per liter. CO₂, 45.7 vols. per cent.

Course: The day of admission she developed a very stiff neck with suggestive Kernig on the right, right deep reflexes increased and right Babinski. The following afternoon she died with failing respirations and weak, irregular heart action. Blood pressure over 300.

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DESCRIPTION OF PLATES

PLATE 177

- FIG. 1. Sarcosporidia in heart muscle fiber. Longitudinal section. $\times 1050$.
 FIG. 2. Sarcosporidia in heart muscle fiber. Cross section. $\times 1050$.

sections stained with phosphotungstic acid hematoxylin. Situated in the center of each of these fibers is an oval body about which no capsule can be demonstrated. This mass is composed of groups of small light blue-staining sickle- or spindle-shaped organisms, measuring approximately $7 \text{ to } 10 \times 2 \text{ to } 2.5$ microns. In the center of each is a darker blue-staining nuclear portion that roughly takes the same form as the organism. In places these are arranged with their long axes pointing more or less in the same direction, although no trabeculae can be seen dividing the whole mass into compartments. Accurate measurements of these bodies cannot be made from the sections because of the difficulty in determining their exact outlines, as they do not lie in a single plane. The cysts themselves measure 82×31 microns.

The above finding is merely an accidental one. There were no symptoms during life which could be referred to this infection. As all the tissue was preserved before the microscopic sections were examined it was not possible to carry out any experimental work on the transmission of this parasite. However, it seems quite probable that it is some form of sarcosporidia, and in a rather early stage of its development, judging from the fact that no capsule can be seen surrounding the cyst. It differs from the majority of species previously described in the following characteristics: (1) In the absence of a surrounding membrane; (2) In the lack of demonstrable septa dividing the cyst into compartments; and (3) In the measurements, which though not carried out on fresh material, indicate that this form is smaller than those previously described. As no sections of skeletal muscle were taken for microscopic examination it is not known whether this infection was general throughout the whole body or merely confined to the myocardium. The case is reported as one of probable sarcosporidial infection of man, and is the second one on record in which the protozoa invaded the heart muscle.

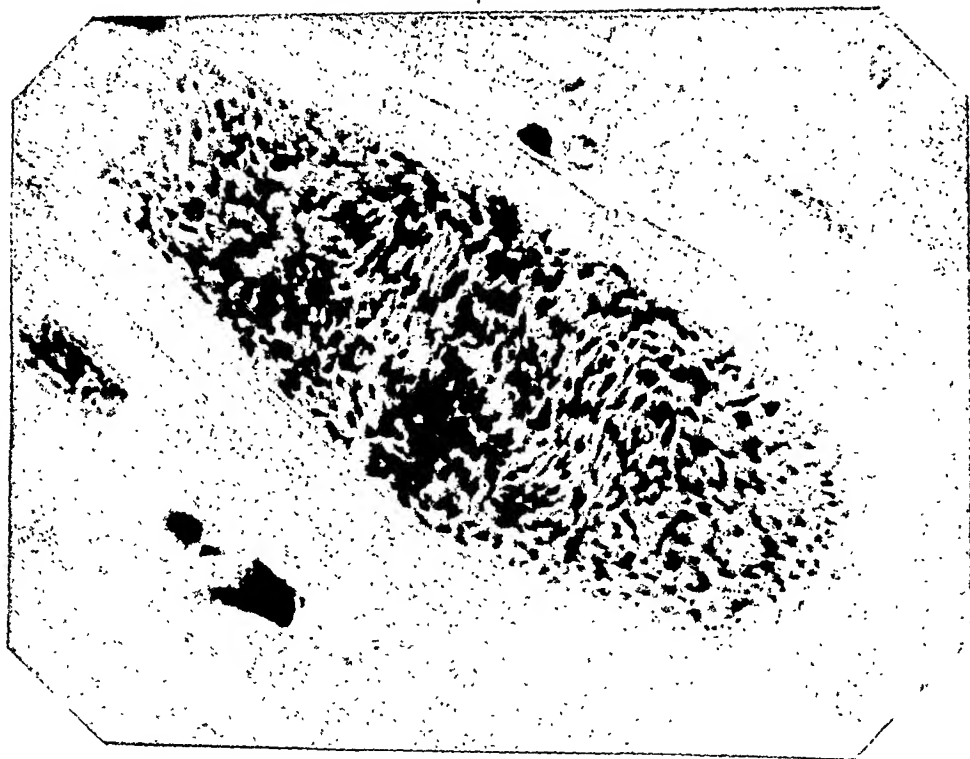
I am indebted to Dr. Walter W. Palmer for permission to transcribe the clinical record of this case.

total defect has taken place in the mucous membrane which will not regenerate. The defect is filled up by granulation tissue which later becomes scar tissue. In time the inflammatory phenomena will disappear. Within this young connective tissue exist remnants of the original tissue including the nerve. The cut nerves regenerate by growing out into the fresh, juicy and flaccid tissue which in time becomes scar tissue or cicatrix containing neuromas of the amputation type.

Are all these alterations which I have described specific for chronic gastric ulcer? In order to answer this I have investigated a large amount of control material, namely, ulcerated cancers of the stomach, cicatrices following gastro-enterostomies, ulcers and cicatrices occurring anywhere in the intestinal tract, and finally typical amputation neuromas of the femoral nerve. In these lesions I have found alterations similar to those already described: perineuritis, nerves embedded in connective tissue and neuromas. No alterations of nerves found in chronic gastric ulcer can be designated as specific if thereby is meant that corresponding changes in their ulcers can be demonstrated. The changes in question are therefore to be regarded as of a secondary nature.

DISCUSSION

Will these secondary nerve changes explain certain symptoms of gastric ulcer? Among the marked symptoms pain is the most predominating. In view of the fact that the nerve branches of the myogastric plexus show almost constant and frequently intense inflammatory changes in the perineural sheath — changes that will create pain, soreness and hyperesthesia in the peripheral nervous system — it seems a justifiable assumption that we are here confronted with at least one of the pain-creating causes. Furthermore, we find exposed nerve branches and nerve cells in the necrotic zone. It is beyond any doubt that the hydrochloric acid will affect them, but how? We do not know the pharmacologic relations intimately enough to be able at present to draw any conclusions with certainty. There is a possibility both of stimulation and of paralysis. Most probably the influence of the acid will be to cause contractions, possibly spasms, and these again may cause pain. The compression exerted on the nerves by the contracting connective tissue in which they are embedded may likewise prove productive of pain.



1



2

A COMPARISON IN NORMAL, THYROIDECTOMIZED AND HYPOPHYSECTOMIZED RATS OF THE EFFECTS UPON METABOLISM AND GROWTH RESULTING FROM DAILY INJECTIONS OF SMALL AMOUNTS OF THYROID EXTRACT *

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Although a very considerable number of reports have been published upon the administration of thyroid to laboratory animals, an interesting and important phase has scarcely been touched upon, for little or no work has been done with the thyroidectomized or the hypophysectomized animal as the test form. The effects of thyroid administration have been studied almost exclusively on the normal animal. Furthermore, except in the experiments of Cameron and Carmichael,^{1,2,3} the amount of thyroid administered has not been in proportion to body weight, the same amount of thyroid having been given throughout each experiment. Consequently, as growth proceeded, the animals received relatively less and less of the gland substance. The results reported from thyroid administration, nevertheless, have been harmonious as regards its effect upon basal metabolism, which is increased, and upon certain organs, the adrenals, kidneys, liver and spleen, which show an enlargement. As regards growth, the results have been more variable, some reporting a retardation, others either no effect or a slight acceleration in the rate of growth.

We have been carrying on for some time the experimental administration of thyroid extract in three types of rats, the unoperated normal, the thyroidectomized and the hypophysectomized. In these experiments fresh thyroid extract has been administered daily by intraperitoneal injection, the dosage given being directly propor-

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tionate to body weight. The intraperitoneal injection of the extract is less time-consuming than feeding, since the animal need not be watched to make certain that the thyroid is all consumed. In these experiments we attempted to give a dosage but slightly in excess of that necessary for a complete replacement therapy in the thyroidectomized animal (*vide infra*), a dosage which we could assume to be "physiologic" for this type. Although direct comparison of the hormonal content of our dosage with that of other investigators is impossible, yet it appears from the physiologic responses of the treated animals that our dosage is less than that heretofore employed. Nevertheless, it gave pronounced results with both thyroidectomized and hypophysectomized animals, effects which have been registered by measurements of basal metabolism, by studies of the specific dynamic action of injected amino acids, and by measurements of general body growth and organ weights.

While the results obtained with each experimental type of rat — the normal, the thyroidectomized and the hypophysectomized — have been of interest in themselves, these results, however, have proved of more interest when compared with effects obtained upon each of the other types. It, therefore, seemed best to bring the data together in one paper so that these comparisons might be emphasized.

METHODS

The completeness of the thyroid ablations has been verified by a careful examination with a binocular dissecting microscope at necropsy and a subsequent study of serial sections through the entire laryngeal region. The hypophysectomies have been done by a method described briefly elsewhere (Smith⁴). A study of serial sections has shown that both the anterior and the posterior portions of the pituitary were completely removed in all instances.

The animals were weighed every three days. Every two weeks they were placed under deep ether narcosis in order to measure accurately their body and tail lengths.

The thyroid extract (suspension) for injection was prepared daily as follows: Fresh sheep thyroids freed from their sheaths were weighed, sterilized for ten minutes in fifty per cent alcohol, rinsed in saline, and ground with sand. They were then diluted in the proportion of 1 gm. of thyroid to 3 cc. of saline, and centrifuged for

twenty minutes. After trying various test dosages it was decided to inject daily, 0.02 cc. of this thyroid extract to each 25 gm. of body weight, a dosage used in all experiments reported here. Although care was used in securing accuracy in dilution and in administration, there was undoubtedly some variability in the amount of thyroid hormone administered from day to day, because of the variability of the hormonal content of the thyroids used in preparing the extract. Such variability was reduced by using glands from several animals for each batch of extract. Any variability, however, in the strength of the extract seemed less objectionable than using a commercial preparation, or even one such as thyroxin which is subjected to severe chemical treatment in its preparation. However, our preference for fresh gland extract rather than thyroxin may be unwarranted, for qualitatively the effects obtained with the injection of the two have been shown to be identical (Cameron and Carmichael³).

The measurements of respiratory metabolism were made with the apparatus recently described by Foster and Sundstroem.⁵ The routine of a metabolism experiment is as follows: The rat, deprived of food for twelve to eighteen hours, is placed in the respiration chamber. Usually within thirty to forty minutes the animal comes to rest and sleeps. Two or three basal periods of twenty minutes each are obtained, after which the animal is taken from the chamber and receives an intraperitoneal injection of glycoll, alanine or glucose.* (The glycoll and alanine are given in 15 per cent solution to the amount of 1.5 grams per kilogram of body weight. Glucose is administered in 30 per cent solution to the amount of 3 grams per kilogram.) On being returned to the respiration chamber the animal becomes quiet, usually within thirty minutes, whereupon measurements are resumed in a series of twenty-minute periods.

The animal chamber is kept at a constant temperature (28.5°–29.5° C.), at which temperature the rat soon becomes drowsy, and in general with our animals not much trouble has been encountered in keeping the subjects quiet. The observer, however, watches the animal and discards any period complicated by movements of the animal.

* In harmony with Weiss and Rapport⁶ but contrary to Liebeschütz-Plaut and Schadow⁷ we find specific dynamic action of glucose and amino acids following parenteral administration.

EFFECTS UPON BASAL METABOLISM

The Unoperated Rat. As shown in Table I, the average of forty-four observations of basal metabolism of normal untreated rats was 4.8 Cal. per kilogram per hour (maximum 5.7, minimum 4.1). This is considerably lower than the figures for the metabolism of fasting rats reported by Abelin⁸ and his co-workers and by Goto.⁹ It may

TABLE I
Summary of Basal Metabolism Experiments

Group	Basal Metabolism					No. of observations
	Cal. per Kg. per hr.*			Mean as per cent of normal untreated animal	Mean of treated animal as per cent of untreated animal of each type	
	Max.	Min.	Mean			
Unoperated not injected	5.7	4.1	4.8	100	100	44
Unoperated injected	6.3	5.3	5.5	115	115	15
Thyroidectomized not injected	4.0	3.2	3.5	73	100	15
Thyroidectomized injected	7.7	5.8	6.4	133	183	15
Hypophysectomized not injected	3.6	2.8	3.2	67	100	7
Hypophysectomized injected	9.1	8.4	8.7	181	272	6

* We have calculated the metabolism on the basis of body weight rather than surface area because of the difficulty in obtaining satisfactory figures for area. We have made careful measurements of skin area of a few animals and have arrived at values widely different from those obtained by Mitchell and Carman²⁴ or by use of Meeh's equation with Rubner's constant for the rat. Our measurements were made as follows: The animal was killed, two girths were marked around the trunk with ink, circumferences were measured at these marks as was length from the nose to the root of the tail. The skin was removed and stretched to these measurements on a sheet of paper, an outline of the skin was traced and the area measured with a planimeter. To this was added the area of the tail (length x circumference at middle) and the feet and ears (estimated). We hesitate to use the value for K derived from our measurements on account of the paucity of our data. However, temporarily assuming the correctness of our value for K we arrive at a basal rate for our normal rats which averages around 40 Cal. per square meter per hour which harmonizes well with Voit's²⁵ classical tabulation of Calories per square meter for animals of various sizes.

be well to point out again that in the work reported here the respiration chamber was maintained at a temperature of 28.5°–29.5° C., that metabolism was measured for short periods, and that only those periods were accepted during which the animal was quiet. The above-mentioned workers used periods of three to seven hours and we do not find that they insisted on complete muscular rest on the part of their subjects. The group of unoperated animals receiving thyroid injections showed (as the mean of 15 observations)

a basal metabolism of 5.5 Cal. per kilogram per hour (maximum 6.3, minimum 5.2). Comparing the mean values for the two groups we find that the thyroid-injected animals average 15 per cent higher than the untreated normals. This stimulating effect of thyroid upon the basal metabolism has been observed many times since the pioneer work of Magnus-Levy (Literature in Grafe¹⁰).

From the data in the literature it seems that the administration of thyroid to normal animals causes only a rather moderate increase in metabolism over a very wide range of dosages. For example, Miyazaki and Abelin⁸ feeding daily 111 mg. of desiccated thyroid to rats found approximately 20 to 25 per cent increase in metabolism; whereas Cramer and M'Call¹¹ with the massive dose of 0.5 gm. dry gland daily to rats, found increases no larger. Our minute dosage yielded an average increase of 15 per cent.

The Thyroidectomized Rat. As is shown in Table 1, the mean of 15 observations of basal metabolism of thyroidless rats receiving no thyroid injections is 3.5 Cal. per kilogram per hour (maximum 4.0, minimum 3.2), while in the thyroidectomized animals receiving thyroid injections the mean of 15 observations is 6.4 (maximum 7.7, minimum 5.8). From this it appears that the effect of thyroid injections is greater on the thyroidectomized animals than on the unoperated group. Thus, whereas thyroid injections increased the basal metabolism of the unoperated animal by 15 per cent, the same therapy applied to the thyroidectomized rats raised the metabolism by 83 per cent. The metabolism of the operated-injected group is even greater than that of the unoperated-injected animals (*i. e.*, mean values of 6.4 and 5.5 Cal. per hour respectively).

Reference to Table 2 shows that our thyroidless rats which received no replacement therapy maintained their subnormal metabolism for at least nine or ten weeks after operation (as long as the experiment was continued). This is not in agreement with the work of Cramer and M'Call¹² who report that the decreased metabolism after thyroidectomy in the rat is only temporary and that after three or four weeks the metabolic rate returns to normal. Such would presumably be the case if the thyroidectomy were incomplete and a fragment of the gland regenerated. That this may be the explanation of Cramer and M'Call's results is a possibility in view of their statement that at necropsy the absence of thyroid tissue was confirmed only by naked eye examination. As shown in

TABLE 2

Basal Metabolism of Thyroidectomized Animals

Designation of animal	Age at operation	Period of no thyroid treatment			Period of thyroid treatment		
		Age at metabolic test	No. of days between operation and metabolic test	Basal metabolism	Age at metabolic test	No. of days between beginning (or stopping) thyr. injections and metab. test	Basal metabolism
		Days		Cal. Kg. hr.	Days		Cal. Kg. hr.
BH 325.....	42	III	69	3.60			
		II4	72	3.47			
					122	2	4.47
					128	8	5.45
W 335.....	40				135	15	5.67
					141	21	5.75
		65	25	3.72			
		67	27	3.67			
W 332.....	40	70	30	3.66			
					120	5	5.44
					124	9	6.49
					131	16	5.26
BH 330.....	42	80	38	3.80			
		88	46	3.74			
BH 318.....	48	97	49	3.35			
		103	55	3.50			
GH 336.....	40	Period of thyroid treatment*			Period of no thyroid treatment		
		67	27	6.78			
B 328.....	42	70	30	6.61			
					131	16	3.97
BH 333.....	40	80	38	5.97			
		88	46	6.04			
					129	10	3.72
		99	59	5.70			
GH 319.....	48				120	7	4.86
					123	10	4.10
		97	49	6.51	125	12	4.02
		103	55	6.30			
W 329.....	42	III	69	6.27			

* Daily thyroid injections were begun immediately after operation.

Table 2 (animals 328, 333, and 336), when the daily thyroid injection of the operated animal was stopped eleven weeks after operation the basal metabolism soon fell from the high level which had been maintained by the thyroid therapy and reached cretinoid levels within two weeks after the injections were stopped, or thirteen weeks after removal of the thyroids. Thus over a period of from ten to thirteen weeks after thyroidectomy there was no indication of the "compensatory mechanism" postulated by Cramer and M'Call in either the treated or the untreated group.

The Hypophysectomized Rat. Our observations on this group, though few, are concordant and a much larger series probably would not significantly change the conclusions that can be drawn from the observations recorded here (Table 1). Seven duplicate determinations of basal metabolism of hypophysectomized rats gave results ranging from 2.8 to 3.6 Cal. per kilo per hour, the mean being 3.2 * (Foster and Smith¹³). To compare with these we have six observations on two hypophysectomized animals receiving daily thyroid injections which showed the remarkably high level of 8.7 Cal. per kilo per hour (max. 9.1, min. 8.4). (These measurements like all those reported here were made while the animal was at rest.) On the basis of mean values, the thyroid injections increased the basal metabolism of the hypophysectomized animal by 170 per cent. As compared with the untreated, normal controls, the increase is 81 per cent. The lowest figure for basal rate in this group is considerably higher than the maximum value in any of the other groups.

EFFECT UPON THE SPECIFIC DYNAMIC ACTION OF AMINO ACIDS AND GLUCOSE

In Figs. 1 and 2 are shown results typical of the specific dynamic action experiments. They are representative of the findings as a whole. In the normal rat following the intraperitoneal injection of glucose or glycocoll there is a prompt rise (within 30 minutes) above the basal rate. This persists for one to two hours and then begins to decrease, disappearing within about three to four hours after the injection. The average of nineteen experiments shows an increase of 16 per cent above the basal for a period of three hours. Similar injections of normal saline do not affect the metabolic rate.

* This very low rate per kilogram in the hypophysectomized animal is not due to large deposits of inert fat. Hypophysectomy does not induce obesity (Smith⁴).

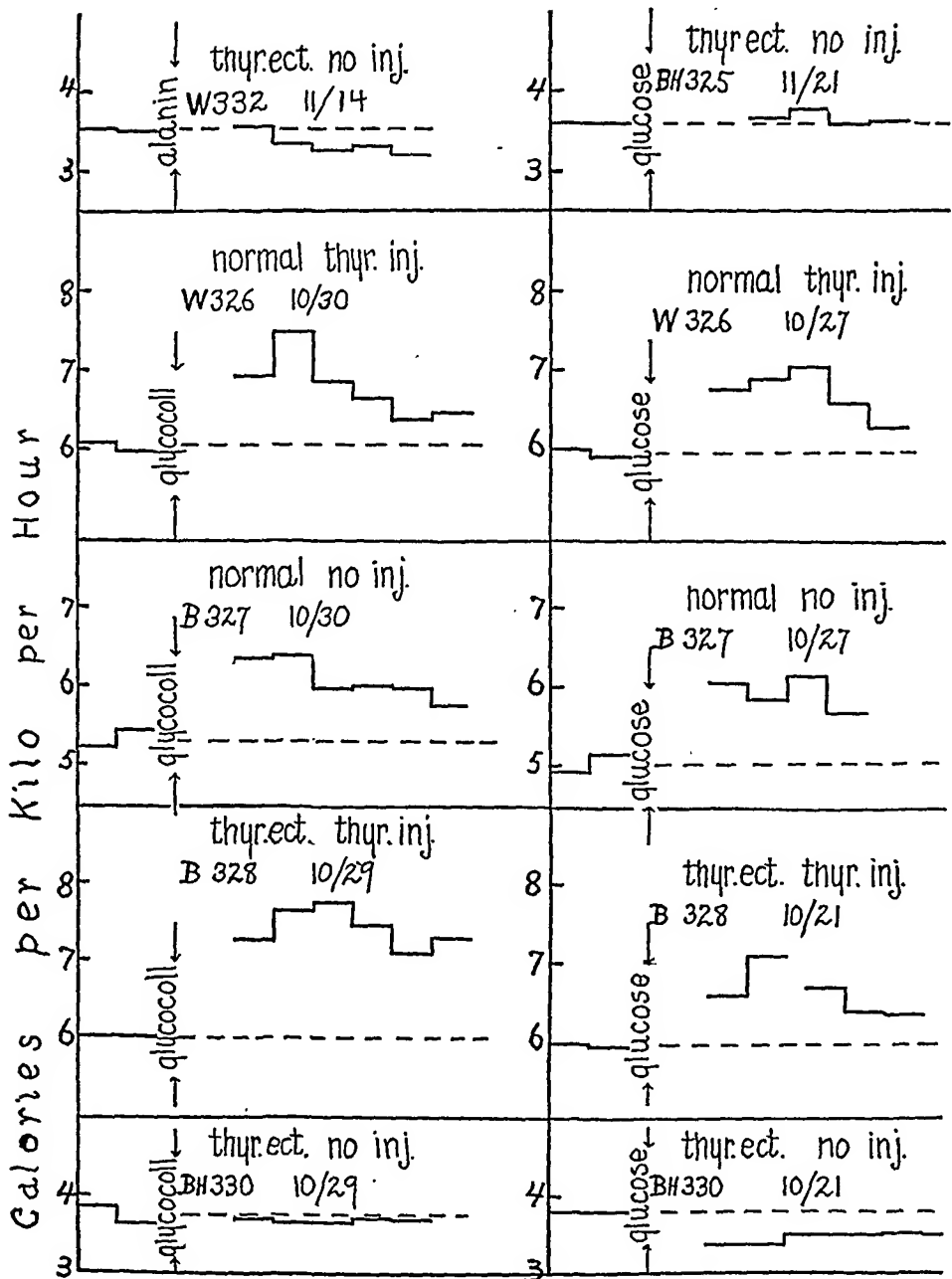


FIG. 1

Metabolism, in 20 minute periods, before and after intraperitoneal injection of amino acids or glucose. The broken line represents the basal rats.

The neuromas, however, play a different rôle. It is a well known fact that central neuromas may be the origin of the neurotrophic ulcers on the extremities. Cutting of the periarterial sympathetic connection allows the ulcer to heal. Wertheimer's⁷ "énervation de l'estomac," cutting of all the nerves that pass to the stomach, has a singularly favorable influence on chronic gastric ulcers. Frequently central cicatrix neuromas will be met with as a secondary nerve alteration in gastric ulcer. Presumably then these neuromas may be the cause of trophic disturbances, thus acting as an additional cause in producing the chronicity of the ulcer.

SUMMARY

1. The frequent occurrence of nerves in the immediate vicinity of gastric ulcer strikingly demonstrates the great power of resistance inherent in nerve tissue.

2. Contrary to what has been shown by previous writers, nerve cells are frequently found in the scar tissue of the ulcer.

3. The inflammatory manifestations are most frequently perineuritis, arising from ascending lymphangitis.

4. The perineuritis extends continuously.

5. The proliferative alterations of the nerve tissue proper appear as central cicatrix neuromas; these may originate in the myogastric plexus and will then contain nerve cells, or they may develop from the periarterial nerves and are then devoid of nerve cells.

6. None of the nerve alterations are specific for chronic gastric ulcer; consequently they may be considered secondary.

7. Perineuritis and the involvement of the nerves in contracting scar tissue may cause pain, the prominent symptom of gastric ulcer. The neuromas may be a contributing cause to the chronicity of the ulcer.

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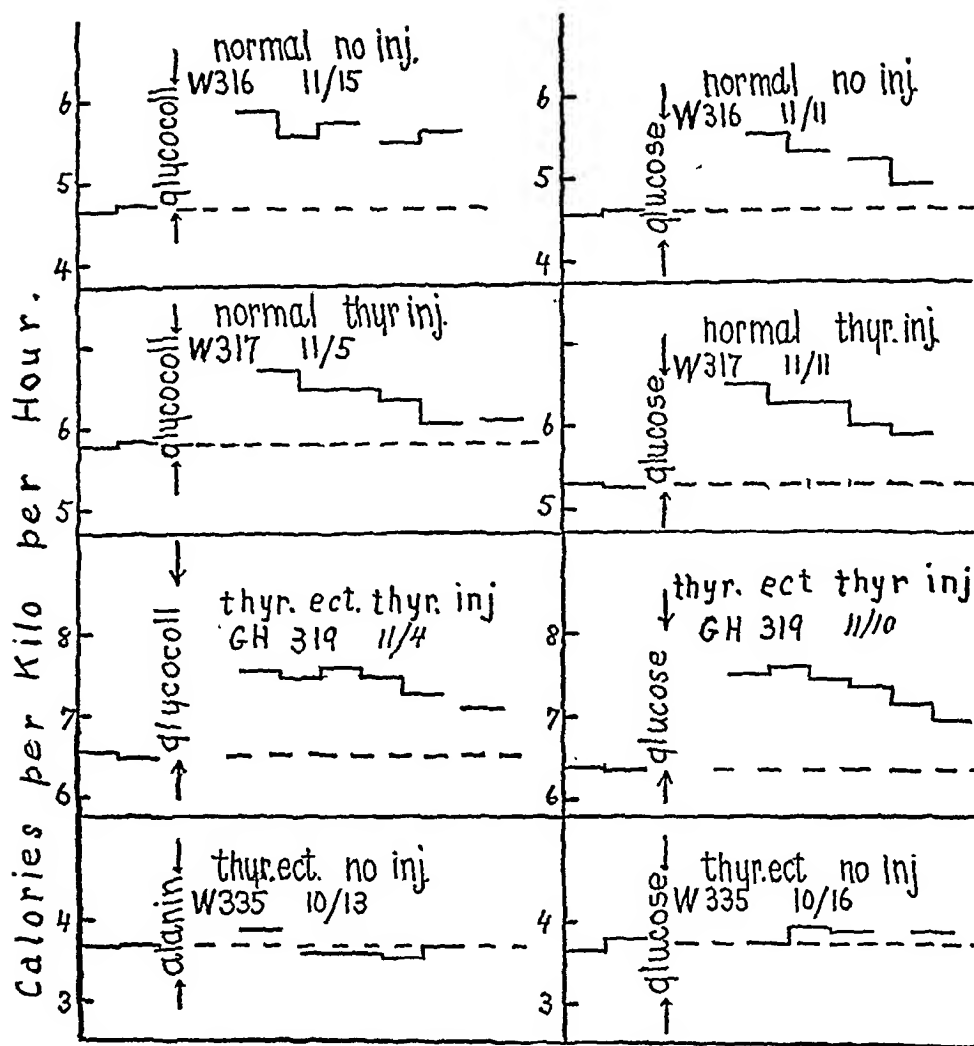
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It will be seen that in animals thyroidectomized some weeks previously the specific dynamic action of glucose or amino acid is uniformly absent, a finding which agrees with those of Baumann and Hunt¹⁴ in thyroidectomized rabbits. In the parallel series of thyroidectomized animals receiving daily injections of thyroid substance, the specific dynamic effect was in full play as was also the case with the unoperated animals receiving thyroid injections.

After several weeks the treatment of the two groups of thyroidless animals was reversed, *i. e.*, the thyroidectomized animals which had been receiving thyroid injections now received none and *vice versa*. Our metabolism data after reversal of the thyroid therapy, though not sufficiently abundant to warrant much discussion, seem to indicate that the animals which had been receiving replacement therapy lost the specific dynamic response within ten days after the thyroid injections were stopped. This is much sooner than one would expect in view of the findings of Baumann and Hunt¹⁴ on rabbits where the specific dynamic action of glucose did not completely disappear until sixty-five days after thyroidectomy.

Animals thyroidectomized for a sufficient length of time to have the specific dynamic response abolished developed the characteristic response to glyocoll injection within fifteen days after thyroid treatments were begun.

As was previously reported (Foster and Smith¹³) no specific dynamic action was observed with the untreated hypophysectomized animals. For the thyroid-injected, hypophysectomized rats we have no satisfactory specific dynamic action experiments. The animals were too restless after the administration of the glyocoll to permit taking accurately the metabolic rate. This point is of interest in connection with the extremely high basal metabolism found in this group of animals (see preceding section). It was possible, by exercising considerable patience, to obtain basal periods during which these animals were quiet, but in the three hours following the injection of glyocoll it was not possible to obtain a single twenty-minute period uncomplicated by movement of the animal, whereas the other animals would often sleep quietly for nearly the whole time.



Time. 20 min. periods.

FIG. 2

Metabolism, as in Fig. 1, before and after amino acid and glucose injections.

TABLE 3
Table Giving the Data for Animals not Represented in Figs. 3 and 4

Designation and Type; Female rats	Operation			Treatment begun *				Experiment terminated				Number days of treat- ment	Change during treatment in		Absolute weight of		Relative per- centage weight	
	Age, days	Weight, gm.	Length, mm.	Substance injected	Age	Weight	Length	Age	Weight	Length	Weight		Length	Adrenals	Ovaries	Adren- als	Ovaries	
W 643 Hypophysectomized	43	114	286	Thyroid	53	103	289	98	108	306	45	5	17	0.0091	0.0120	.38	.45	
W 644 Hypophysectomized	43	110	284	Thyroid	53	98	288	98	101	303	45	3	15	0.0081	0.0096	.37	.38	
B 646 Hypophysectomized	43	132	288	Thyroid	53	109	290	98	110	305	45	1	15	0.0093	0.0160	.38	.59	
B 645 Normal.....	43	122	296	None	53	148	316	98	206	364	45	58	48	0.0453	0.0508	1.00	1.00	
B 647 Normal.....	43	110	273	None	53	129	296	98	185	356	45	56	60	0.0483	0.0531			
W 159 Thyroidectomized	71	146	340	None	75	148	340	182	185	351	107	37	11	0.0240	0.0387	.56	.70	
GH 160 Thyroidectomized	71	150	340	None	75	148	340	182	170	350	107	22	10	0.0202	0.0377	.50	.77	
BH 162 Thyroidectomized	71	142	334	Thyroid	75	142	334	182	204	382	107	62	48	0.0501	0.0603	1.05	1.02	
GH 161 Normal.....	71	149	339	Thyroid	75	151	339	182	237	395	107	86	56	0.0522	0.0686	.94	1.00	
GH 158 Normal.....	71	182	354	None	75	179	354	182	220	396	107	41	42	0.0515	0.0639	1.00	1.00	
BH 318 Thyroidectomized	48	104	291	None	52	114	293	124	151	314	72	37	21	0.0227	0.0399	.68	1.13	
GH 319 Thyroidectomized	48	103	283	Thyroid	52	109	285	124	195	355	72	86	70	0.0511	0.0474	1.36	1.04	
W 317 Normal.....	48	101	290	Thyroid	52	109	291	124	201	374	72	92	83	0.0606	0.0623	1.36	1.32	
W 316 Normal.....	48	101	292	None	52	111	293	124	212	378	72	101	85	0.0470	0.0496	1.00	1.00	
W 151 Thyroidectomized	83	182	349	Thyroid	87	178	349	194	253	391	107	75	42	0.0583	0.0593	1.12	1.02	
W 153 Normal.....	83	173	354	Thyroid	87	175	354	194	222	395	107	47	41	0.0496	0.0654	1.08	1.31	
BH 154 Normal.....	83	174	353	None	87	175	353	194	217	394	107	42	41	0.0447	0.0489	1.00	1.00	

* For comparison the weight and age of the animals receiving no treatment is also given in this column.

EFFECTS UPON GROWTH

The Normal Unoperated Rat. Reference to Table 3 shows that our thyroid dosage did not alter in any marked degree either the general body weight or the length of normal animals, as compared with their untreated littermate controls. The variations exhibited are clearly within the normal limits. Growth inhibition from thyroid administration has been reported by Cameron and Carmichael,^{1, 2} and Gudernatch¹⁶; while no influence or a slight increase in the rate of growth is reported by Herring,^{17, 18} Hoskins¹⁹ and Schäfer.²⁰ That this difference in the effects upon growth is due to the amount of thyroid administered has been clearly shown by the work of Herring¹⁸ Hewitt²⁶ and Cameron and Carmichael.¹ It appears that our dosage is too small to influence body weight or skeletal growth. An examination of the adrenal weights of our thyroid-injected normal animals shows in but one case any increase over the normal. In this case the increase in weight is not great enough to appear of significance. These results are not in harmony with the findings of other investigators; the adrenal hypertrophy, induced by thyroid feeding, reported by R. G. Hoskins²² having been uniformly confirmed. This failure of our thyroid dosage to cause any alteration in the rate of general body growth or in the weights of the adrenals is here emphasized for it shows the minuteness of our dosage. Nevertheless, this small dose when given to the thyroidless rat exerted a profound effect as will be shown in the next section.

The Thyroidectomized Rat. Although the first successful replacement therapy was achieved in operative myxedema or endemic cretinism, experimental investigators appear not to have attempted a replacement therapy in the thyroidectomized animals. The effect of thyroidectomy, however, has been thoroughly studied by a number of investigators who have reported, among other effects, a retardation in the rate of general body growth and a diminution in the weight of adrenals. Our thyroidectomized animals, of which we had quite a large series, showed these characteristic effects. Since our data upon this point are harmonious with that given by other investigators it seems unnecessary to present it here. We have pointed out above that our small thyroid dosage failed to elicit any appreciable response in the unoperated animals. In contrast to this is the striking response elicited in the thyroidectomized

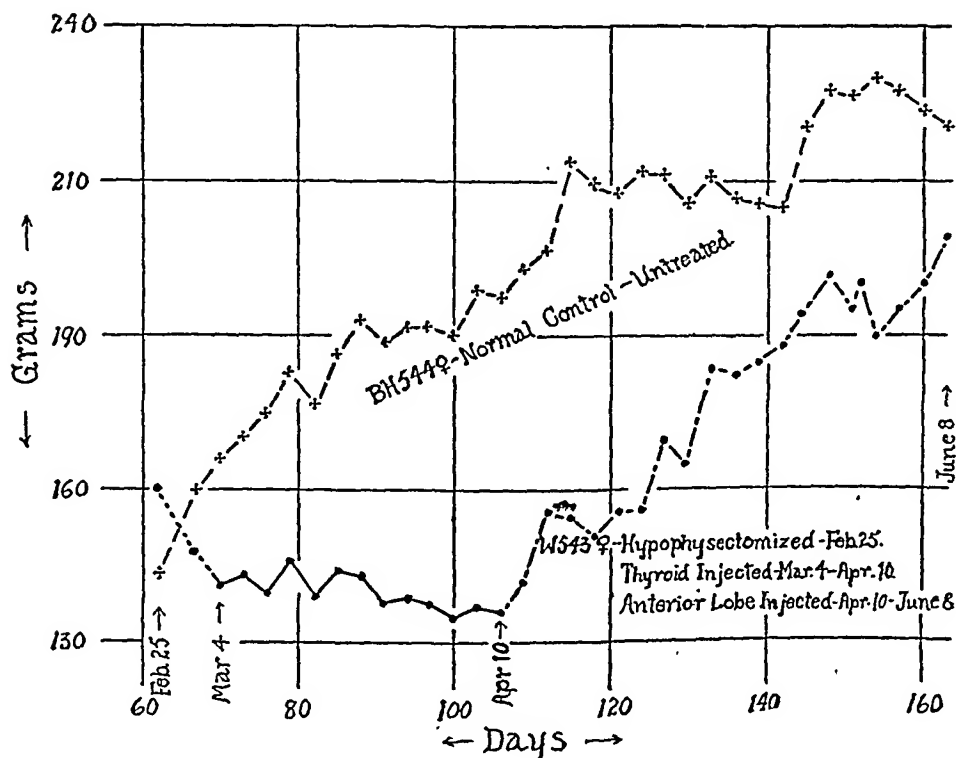
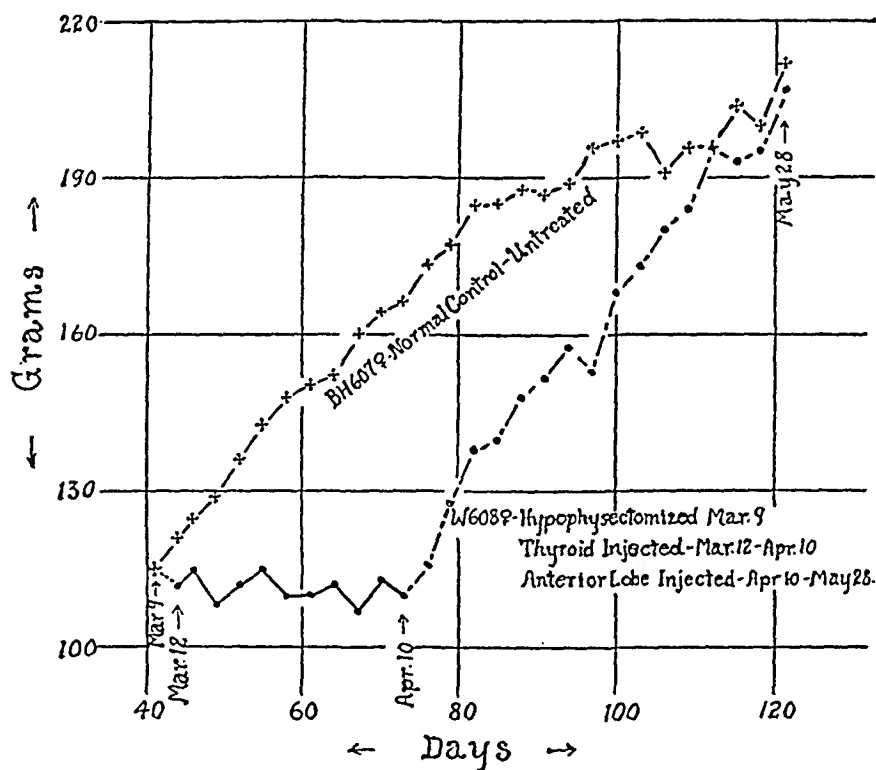


FIG. 4

Curves showing body weights of two hypophysectomized rats and the littermate controls. The former received thyroid injections during the first part of the period, followed by anterior pituitary injections. The controls received no treatment.

animal by this same dosage. It brought about a normal growth rate in these animals which prior to treatment were growing slowly or not at all. It thus had a pronounced effect upon growth both as regards body weight and total length (Table 3, Fig. 3). The adrenals also, at necropsy, were of normal size and structure in these treated animals, thus showing the restorative effects of this treatment upon these glands. It seemed to us a striking example of the

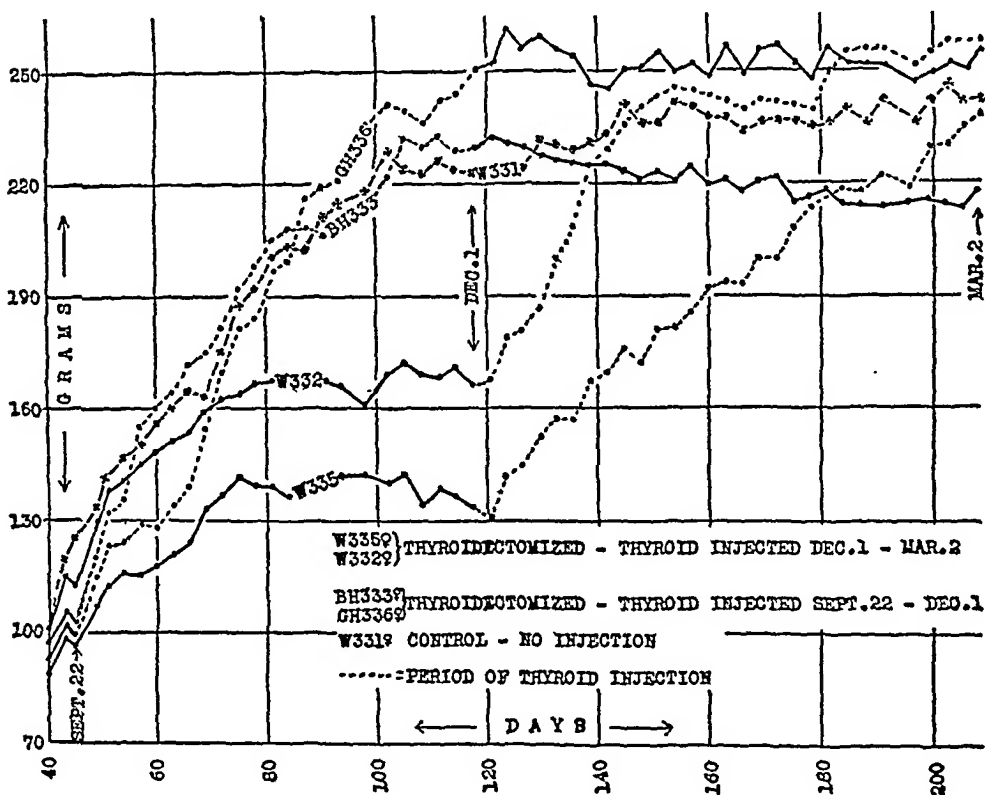


FIG. 3

Curves showing body weight of four thyroidectomized rats and one control, all littermates. Two of the thyroidectomized animals (BH333, GH336) received thyroid injections during only the first half of the period, the other two (W332, W335) during only the last half.

utilization of a secretory product when a physiologic deficit exists. That the normal animal was not affected by this dosage shows the stability of the balance which exists among the endocrine glands and the capacity of the normal animal to withstand an unusual amount of thyroid secretion.

The Hypophysectomized Rat. In five hypophysectomized rats (Table 3, Fig. 4) we have injected over a considerable period of time the same thyroid dosage in proportion to body weight given to

tomized as compared with the normal rat. An avascularity and a smaller size of the thyroids in thyroid-treated normal rats (Cameron and Sedziak ¹⁵) may form a part of the mechanism by which the amount of the thyroid hormone may be controlled as has been suggested by these investigators. That it does not form the entire mechanism for such a control appears from the determinations of the basal metabolism in the thyroidectomized and the hypophysectomized rat receiving thyroid treatment. Although the amount of thyroid administered was identical, in proportion to body weight, yet the rise in the metabolic rate of the thyroidless was considerably greater than it was in the normal rat, and the response of the hypophysectomized animal was even more profoundly increased. This greater rise of the thyroidless and hypophysectomized animal as compared with the normal, cannot be referred to any compensatory inactivity of the thyroids in the operated animals for in one the thyroids were absent and in the other they were atrophied.

Our work also clearly shows that in the absence of the hypophysis growth cannot be brought about by thyroid treatment. It would seem that the growth stimulation induced by thyroid administration in the thyroid dwarf is due to a stimulation in the secretory activity of the hypophysis. This conclusion is in harmony with the findings of Trautman who interpreted the hypophyseal changes following thyroidectomy as degenerative in nature and with those of Livingston ²³ who found that thyroid feeding prevented these changes. It is supported also by the work of Flower and Evans who found that injection of a suspension of bovine hypophysis induced growth in the thyroidless rat, and by the fact that injection of this bovine pituitary suspension or pituitary transplants induced growth in the hypophysectomized rat (Smith ⁴).

Our experiments, in common with those of many other investigators, show the stimulating effect of thyroid administration upon the basal metabolism of the normal animal. It is of interest to compare the effect upon metabolism with the size of the dose of the thyroid given. Miyazaki and Abelin ⁸ fed from 75 to 110 mg. of desiccated thyroid daily to rats of approximately 150 gm. weight and recorded increases in basal metabolism of from 15 to 25 per cent. From the data reported by Cramer and M'Call ¹¹ it is difficult to make definite recalculations, but in most cases the increase in metabolism induced by the massive dose of 500 mg. of dry gland daily to a 150

the normal and to the thyroidectomized animal. This treatment neither stimulated growth in animals whose growth had been stopped by pituitary ablation nor did it repair the endocrine organs and the genital system which atrophy as a result of hypophysectomy (Smith ⁴).

That the failure of the thyroid treatment to stimulate general body growth or growth of the adrenals and the genital system is not due to any incapacity of these animals to respond to treatment is shown by the fact that daily anterior pituitary transplants stimulate their general body growth and have a marked restorative effect upon their atrophied adrenals, thyroids and genital system (Smith ⁴). Daily intraperitoneal injections of a fresh bovine anterior pituitary suspension also stimulates their general body growth although it does not restore the atrophied endocrine organs or genital system. In order to contrast the absence of any growth stimulation from thyroid administration with the growth stimulation given by the anterior pituitary we present in Fig. 4 the growth curves of animals which were, for a period, injected with thyroid, this being followed by a period in which injections of a bovine anterior pituitary suspension were given.

The failure of thyroid administration to induce growth in these pituitaryless animals is in accord with results obtained in clinical cases of pituitary dwarfs, in which thyroid treatment is ineffective. In the absence of the pituitary secretion the thyroid cannot induce growth.

DISCUSSION

We have shown that injections of thyroid extract in minute doses, given in proportion to body weight, give, as regards basal metabolism, growth, and organ weights, very different effects in the different groups of animals studied.

A thyroid dosage which does not appreciably alter the growth rate or the adrenal weights of unoperated controls induces a normal increase in body weight, in total length and in the weights of the adrenals in rats stunted by thyroidectomy. The thyroid-injected, thyroidectomized animals thus markedly exceeded in body weight and in the weight of their adrenals their uninjected, thyroidless, littermate controls. We have no explanation which satisfies us for this difference in the response to thyroid injection of the thyroidec-

Normal rats gave no growth or marked adrenal response to these injections.

Thyroidectomized rats, on the other hand, gave a pronounced growth and adrenal response. Their rates of growth and adrenal weights consequently greatly exceeded those of their untreated thyroidectomized littermates.

Rats which had been stunted by hypophysectomy showed neither growth response to the injections nor restoration of their atrophied adrenals, thyroids, or genital systems. This failure of the thyroid injections is not due to any incapacity of these animals to respond to treatment, for pituitary transplants cause them to grow and have a marked restorative effect upon their atrophied endocrine and genital systems. In the absence of the anterior pituitary secretion, the thyroid cannot stimulate growth.

Thyroid injections have much greater effect upon the basal metabolism of thyroidectomized and still greater effect upon that of hypophysectomized rats than upon that of unoperated animals.

In agreement with Baumann and Hunt,¹⁴ the specific dynamic action of glucose, glycocoll and alanin is absent in thyroidectomized rats six weeks or more after operation. In thyroidless rats receiving replacement therapy, specific dynamic action is in full play.

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gm. rat is less than 25 per cent. Our dosage prepared and administered as described under "Methods" would correspond to about 8 mg. of dry gland substance for a 150 gm. rat if we assume fresh sheep thyroid to contain 75 per cent water. This dosage resulted in an average increase of 15 per cent above the normal basal level. In the reports of the above-named workers we have no assurance that the whole dose of thyroid was in each case consumed and a strict comparison of their dosages with ours is probably not possible. However, it seems that large doses have relatively less effect than do small and that the normal organism is in some manner well "buffered" against excessive amounts of thyroid secretion. Whatever this hypothetical "buffering mechanism" may be, it appears to be less effective in the thyroidectomized and far less effective in the hypophysectomized animal, as can be shown by a comparison of the actual increase in Cal. per Kg. per hour of the treated over the untreated animal of each type. Thus, in the unoperated animal the mean increase induced by thyroid injections was 5.5 less 4.8 or 0.7 Cal. per Kg. per hour; in the thyroidectomized group the increase was 6.4 less 3.5 or 2.9 Cal. per Kg. per hour and in the hypophysectomized animal 8.7 less 3.2 or 5.5 Cal. per Kg. per hour. That is, the *increase* in basal heat production caused by thyroid injections was 4.1 times greater in the thyroidectomized and 7.9 times greater in the hypophysectomized than in the unoperated animal.

Our experiments showing the absence of specific dynamic action of amino acids and glucose in thyroidectomy confirm the work of Baumann and Hunt.¹⁴ They do not, however, lead to any explanation of the nature or cause of specific dynamic action. It is not at all likely that there is a qualitative difference in the manner in which these substances are metabolized by thyroidless animals. It seems more probable that it is a matter of reaction velocities. It is planned to compare the rates of absorption and the completeness of utilization of glucose and amino acids by normal and thyroidectomized animals.

SUMMARY

Minute doses of a saline extract (suspension) of fresh sheep thyroids were injected daily into normal, thyroidectomized or hypophysectomized rats. The dosage was given in proportion to body weight, 0.02 cc. being given for each 25 gm. body weight.

DESCRIPTION OF PLATES

PLATE 10

FIG. 1. Preparation 12. Section through nerves near ulcer, each surrounded by tunics of transformed peripheral neuroglia. Section stained by Masson's three-color method. About $\times 160$.

PLATE 11

FIG. 2. Preparation 15. Transverse section through lesser curvature. In upper left-hand corner, remains of mucous membrane; in lower right-hand corner, arteries entering muscular coat. Wall of stomach crammed with nerve bundles of every size. About $\times 40$.

FIG. 3. Preparation 3. Section through neuroma derived from the myogastric plexus and containing numerous nerve cells. The black stripes are muscle fibers. Stained by Masson's method. About $\times 120$.

PLATE 12

FIG. 4. Preparation 2a. Periarterial neuroma near base of ulcer. The arterioles are subject to considerable pathologic changes. Stained by Masson's method. About $\times 80$.

PLATE 13

FIG. 5. Preparation 9. Detail of neuroma derived from myogastric plexus, showing the extremely irregular course of the individual branches. Note the inflammatory phenomena. Stained by Van Gieson-Hansen's method. About $\times 300$.

FIG. 6. Same preparation as in Fig. 4. Detail of neuroma within the cicatricial zone. The nerve fibers are interlacing the dense connective tissue. Stained by Masson's method. About $\times 120$.

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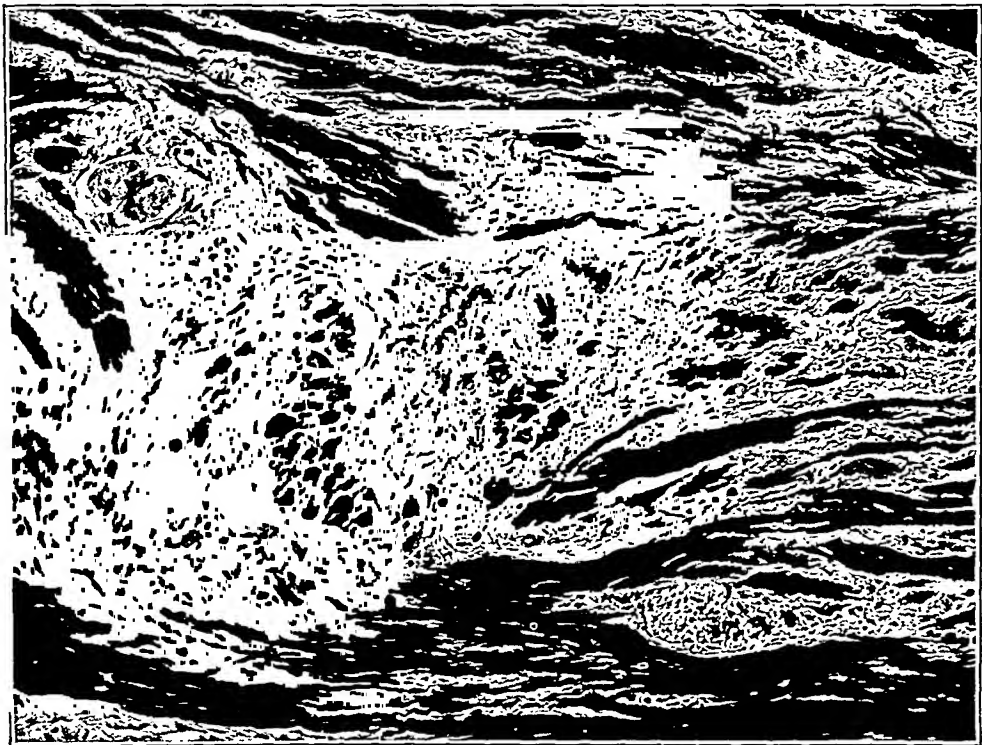
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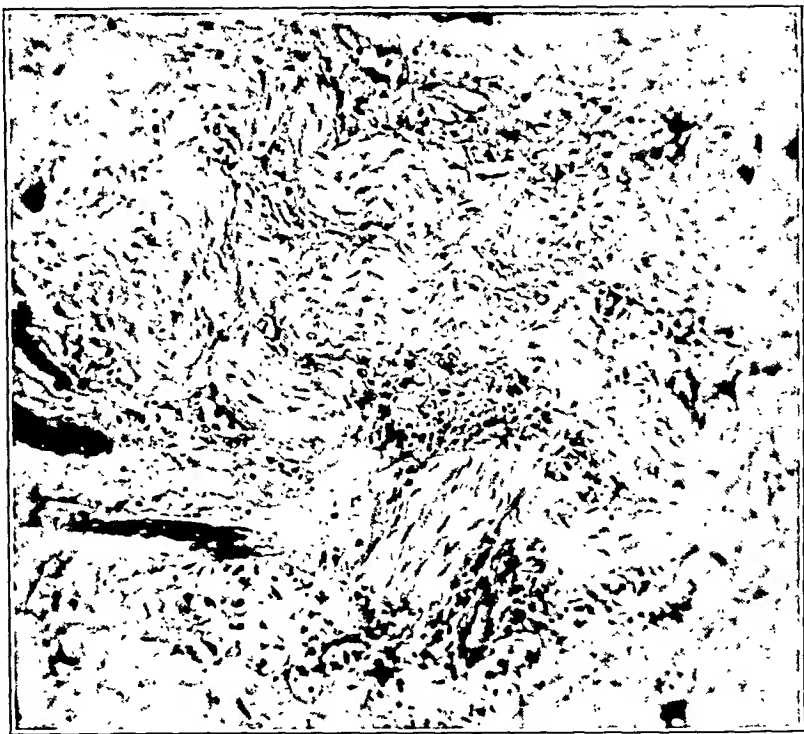


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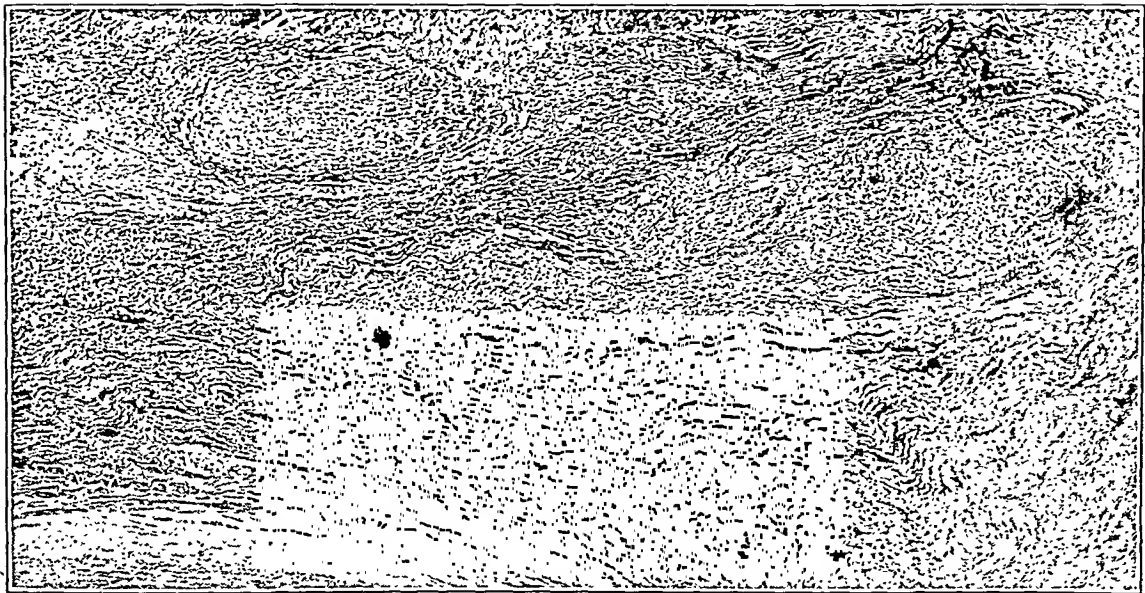


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The specimen was received within a few minutes after removal at operation and thin sections of tumor tissue were placed immediately in Zenker's fluid and other fixatives.

Microscopic Examination. The large tumor proves to be rapidly growing and at its periphery is infiltrating the muscle of the uterus. It is composed in places of spindle-shaped cells provided with very fine straight or slightly curving fibroglia fibrils. Between the cells run delicate wavy collagen fibrils in small numbers. These two types of fibrils demonstrate that the type cell of this tumor is the fibroblast. In other fields the cells vary from oval to spherical and few or no distinct fibrils can be found in connection with them. In still other areas the collagen fibrils are numerous and fused into thick hyaline strands which form a meshwork with the cells filling the spaces. These areas evidently represent the older parts of the tumor and have undergone retrograde changes.

Mitotic figures are present in great numbers in all areas but especially in those in which the tumor is growing most rapidly. Some of the mitoses are single but many are multiple and as a result giant cells containing large lobulated or multiple nuclei surrounded by delicate, lightly staining cytoplasm are numerous. Still more striking is the presence of many large foreign body giant cells often with one to two dozen or more nuclei embedded in rather dense cytoplasm. They occur not only singly but also in groups of various sizes. In places are small cavities, some lined with these giant cells and filled with serum, while others are filled with giant cells and look like small abscesses.

These foreign body giant cells are evidently at work dissolving the hyaline stroma left in places owing to necrosis of the tumor cells which formed it. In many areas the giant cells can be found closely applied to the intercellular substance. In this situation they have developed at the surface of contact a border of delicate short rods resembling cilia which stain intensely with eosin in eosin-methylene blue preparations of Zenker-fixed tissue and a deep blue with phosphotungstic acid hematoxylin. A similar layer of minute rods has been observed on the surface of osteoclasts where they are applied in the lacunae to bone which is undergoing solution. They may represent changed centrosomes as none can be found in these giant cells although they are present in the tumor cell type.

The foreign body giant cells seem to be formed entirely by fusion

TUMOR AND FOREIGN BODY GIANT CELLS IN A FIBROSARCOMA OF THE UTERUS *

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AND

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The main purpose of this brief communication is to put on record, chiefly photomicrographically, a rapidly growing fibrosarcoma of the uterus containing two different types of giant cells in great numbers. This combination in a marked degree is rarely seen except in tumors arising in bone.

There are at least three types of giant cells which it is important to recognize and distinguish clearly from each other. The first results from multiple mitosis and occurs most often in rapidly growing tumors but occasionally under inflammatory conditions. The second is due to fusion of endothelial leucocytes to accomplish work which single cells are unable to perform. It is found in association with foreign bodies of all sorts such as lime salts, cholesterin and fatty acid crystals, cornified epithelial cells, and sutures and other foreign bodies introduced into the body. The third is formed by enlargement and direct division of nuclei and occurs in various cells under conditions of degeneration as in the epidermis, for example, following repeated freezing.

The tumor to be reported here illustrates the first two types of giant cells which are much more common and important than the third.

Gross Examination. The specimen consists of a symmetrically enlarged but somewhat nodular uterus measuring $15 \times 8 \times 10$ cm. On section the lumen is filled with foul smelling, necrotic, greenish tags of tissue which are continuous with an apparently walled off, moderately solid, whitish tumor mass measuring $10 \times 5 \times 5$ cm. Some of the necrotic material is calcified. In addition to the large mass there are multiple, small, dense, white nodules elsewhere in the uterine wall, evidently ordinary leiomyomas.

* Received for publication November 1, 1926.

so far as they themselves are concerned. All they indicate is an attempt to dissolve something which the endothelial leucocytes individually are incapable of accomplishing. They, therefore, signify an attempt at repair.

SUMMARY

A fibrosarcoma of the uterus containing tumor and foreign body giant cells in great numbers is reported.

The tumor giant cells are formed from multiple mitoses and indicate rapid growth and malignancy.

The foreign body giant cells are evidently due to fusion of endothelial leucocytes attracted into the tumor by the intercellular substance left by necrosing tumor cells. The leucocytes are attempting to dissolve the hyaline collagen and have fused for this purpose. Along the surface where they are applied to it a layer of minute rods is formed, which are perhaps altered centrosomes. Osteoclasts present the same structure.

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2. Cohen, Mortimer. Observation of formation of giant cells in turtle blood cultures. *Am. J. Path.*, 1926, ii, 431.
3. Lewis, W. H. Giant cells of the blood. *Science*, 1926, lxiv, Supplement x.

DESCRIPTION OF PLATES

PLATE 14

FIG. 1. Spindle cell portion of tumor showing delicate fibrillar intercellular substance between the cells. Three mitoses present. $\times 500$.

FIG. 2. An older part of the tumor; the intercellular fibrils are fused to form a hyaline meshwork. Mitoses numerous. $\times 500$.

PLATE 15

FIG. 3. Two mitoses, one large, the other of normal size; also tumor and foreign body giant cells. $\times 750$.

FIG. 4. A multiple mitosis and tumor and foreign body giant cells. $\times 1000$.

PLATE 16

FIG. 5. A large group of foreign body giant cells. $\times 100$.

FIG. 6. Several foreign body giant cells; one large tumor giant cell at right. $\times 500$.

of endothelial leucocytes which have been attracted into the tumor by the intercellular substance left by necrosing cells. All stages in their growth from small to large giant cells can be followed. Leucocytes adjoining a giant cell are first surrounded by cytoplasmic processes and then included within the cell. At first they stand out distinctly in the cytoplasm being surrounded by a light zone but later they fuse completely with it. Not infrequently an endothelial leucocyte in mitosis is taken into a giant cell in the same way and completes its cycle of development there before fusing with the cytoplasm.

The most striking feature of this case is the presence of the great number of foreign body giant cells in a tumor not involving bone in any way and due apparently to the attraction exerted on endothelial leucocytes by collagen which requires removal by solution in the same way that bone does. On this account, one point which has to be considered is the possibility that the attraction exerted for endothelial leucocytes in this tumor by the hyaline collagen is due to a deposit of lime salts in it. Calcification of the necrotic part of the tumor was noted on fresh examination but no evidence of lime salts could be found in sections taken from the living parts of the tumor and preserved in an alcohol-formalin mixture, but no silver test for calcium was made.

A second point of interest in this tumor is that it is easily possible to trace the formation and development of the foreign body giant cells by observing the way in which they not only fuse with one another but are incorporated into giant cells already formed. It is perfectly analogous to the formation of giant cells around agar injected subcutaneously as reported by Forbes¹ many years ago.

Recently, Cohen² and Lewis³ have reported that they have been able to follow the formation of giant cells from endothelial leucocytes directly under the microscope in cultures of the blood.

Tumors of the uterus containing giant cells have been reported by several observers during the past few years and attempts have been made to draw prognostic conclusions from the presence of such giant cells without, however, precise statement of the nature of the cells, *i. e.*, whether they are true tumor or foreign body giant cells. The distinction is very important because multinucleated tumor cells indicate rapid growth and therefore marked malignancy while those of the foreign body type have an entirely opposite significance

PLATE 17

FIG. 7. A large foreign body giant cell in center applied to collagen which it is dissolving. A crescent of short rods at line of junction. $\times 750$.

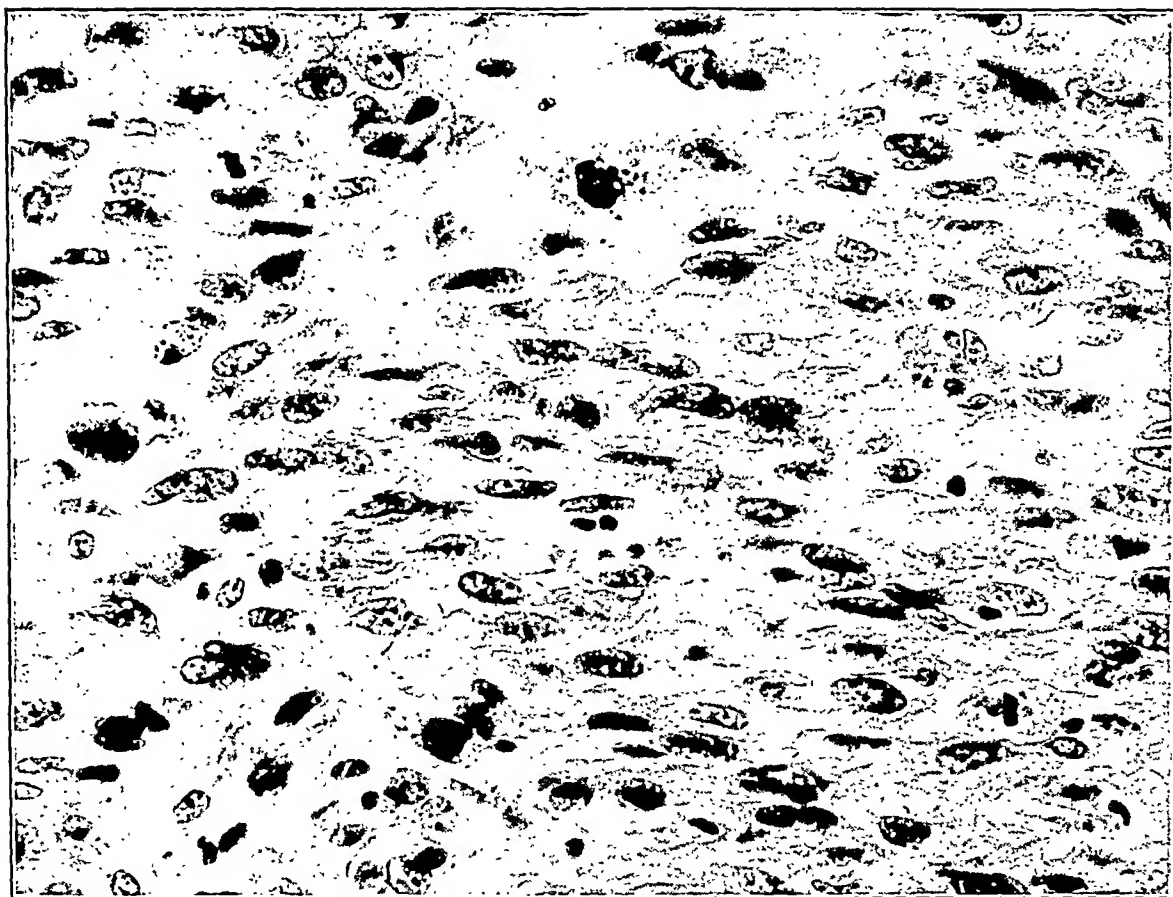
FIG. 8. A giant cell on right almost encircling a mass of collagen. Line of junction only moderately well defined by minute rods. On left, three foreign body giant cells, a large mitotic figure and a tumor giant cell. $\times 750$.

PLATE 18

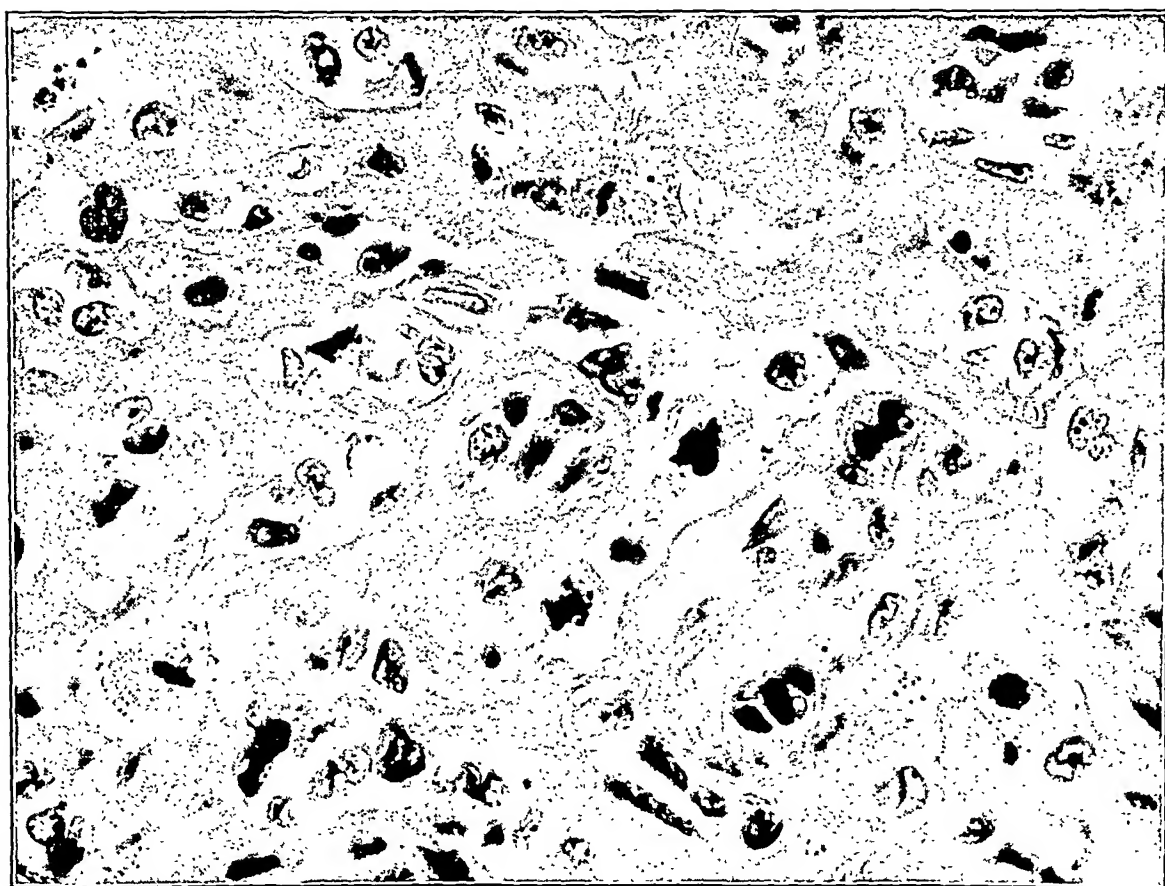
FIG. 9. A foreign body giant cell which has incorporated two endothelial leucocytes, one in mitosis. $\times 750$.

FIG. 10. An endothelial leucocyte in mitosis in a foreign body giant cell. $\times 750$.

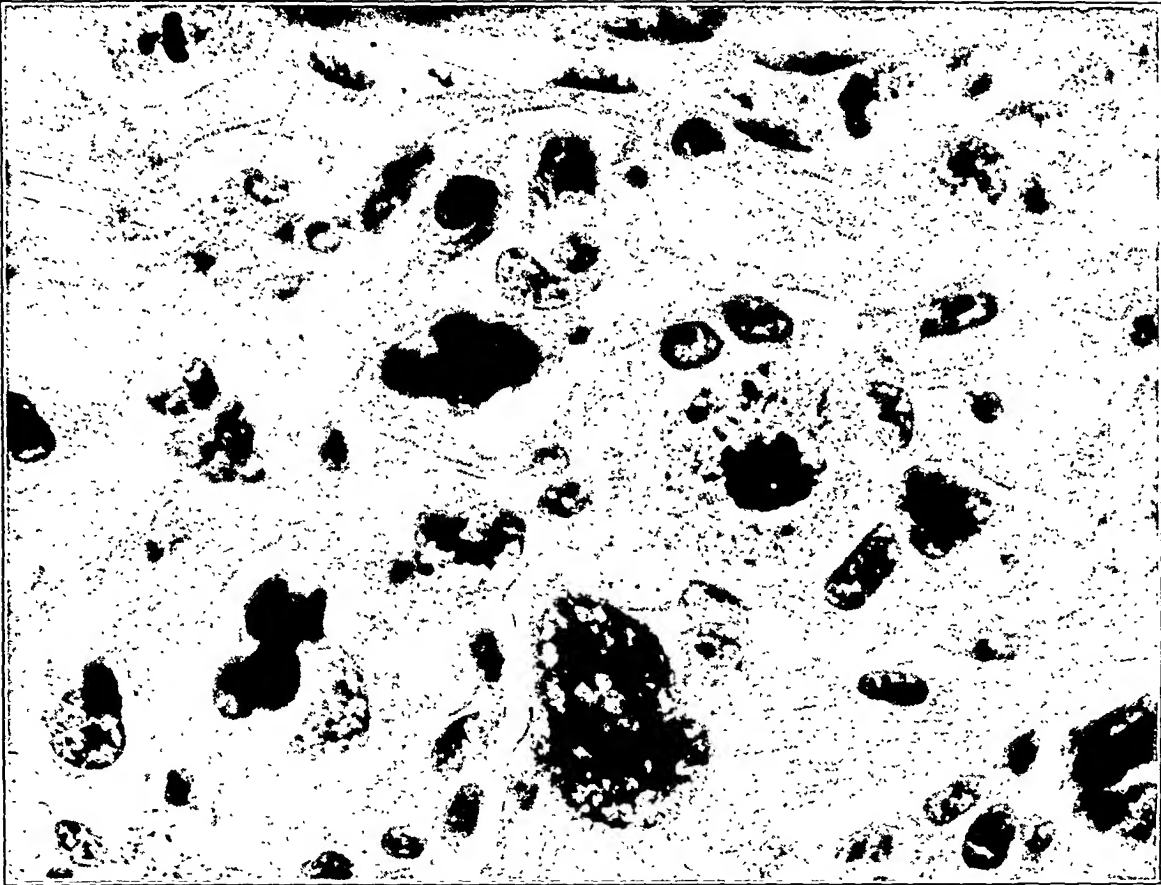
FIG. 11. Endothelial leucocytes, of which one is in mitosis, just beginning to fuse around the remains of a small mass of agar injected subcutaneously. $\times 1000$.



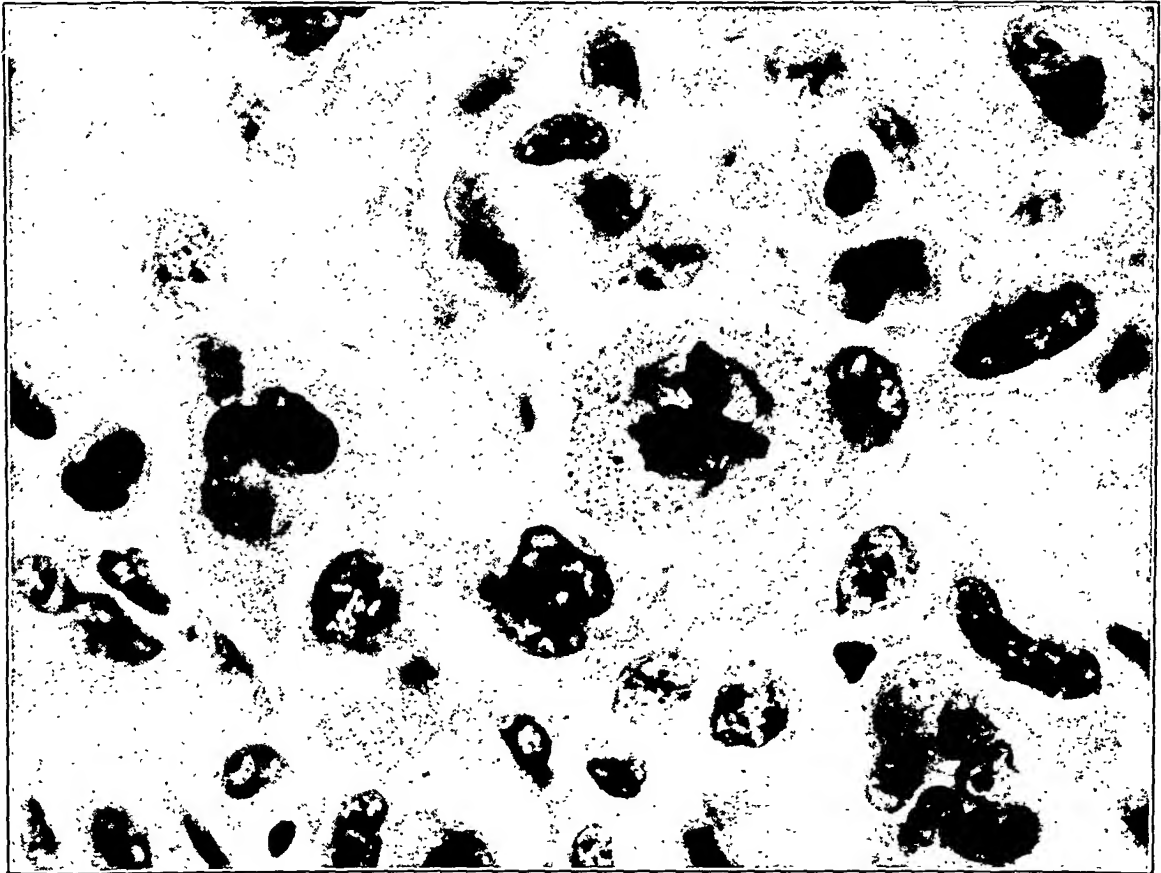
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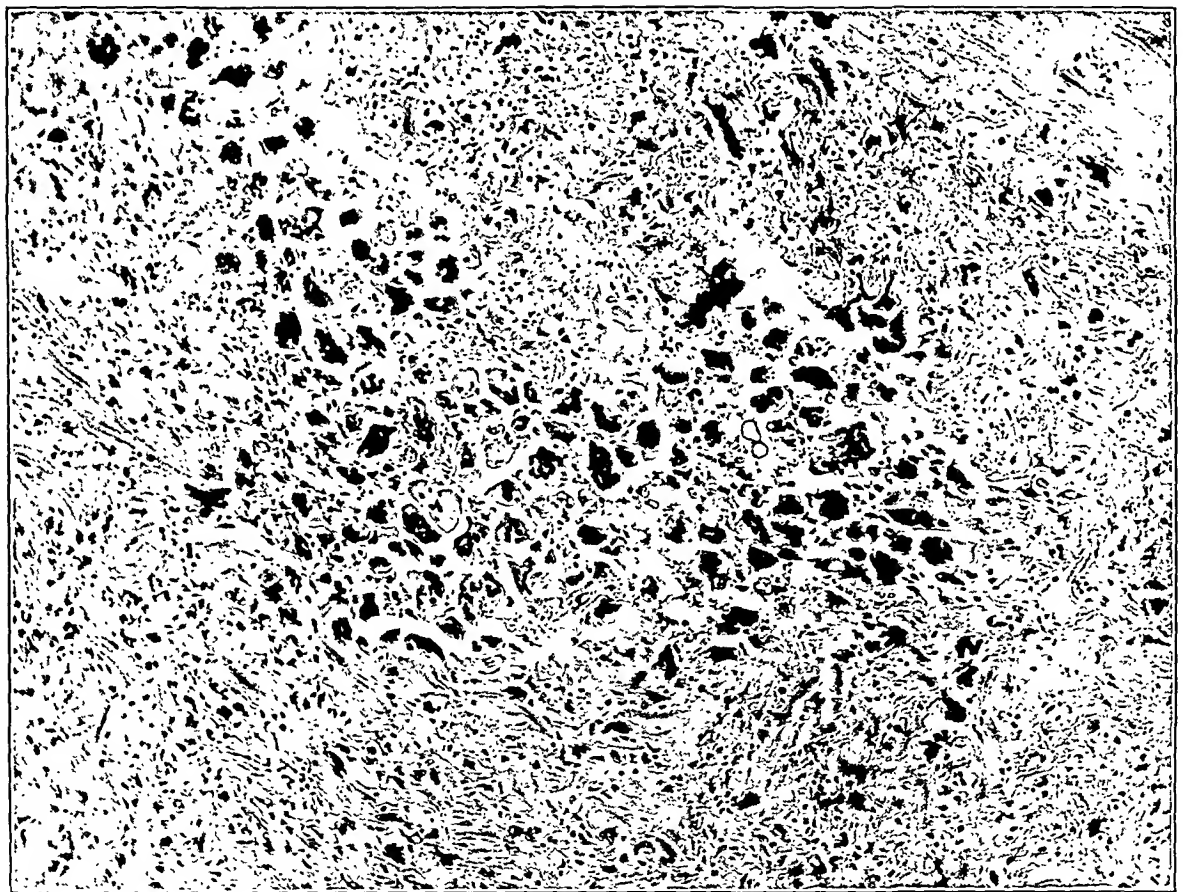
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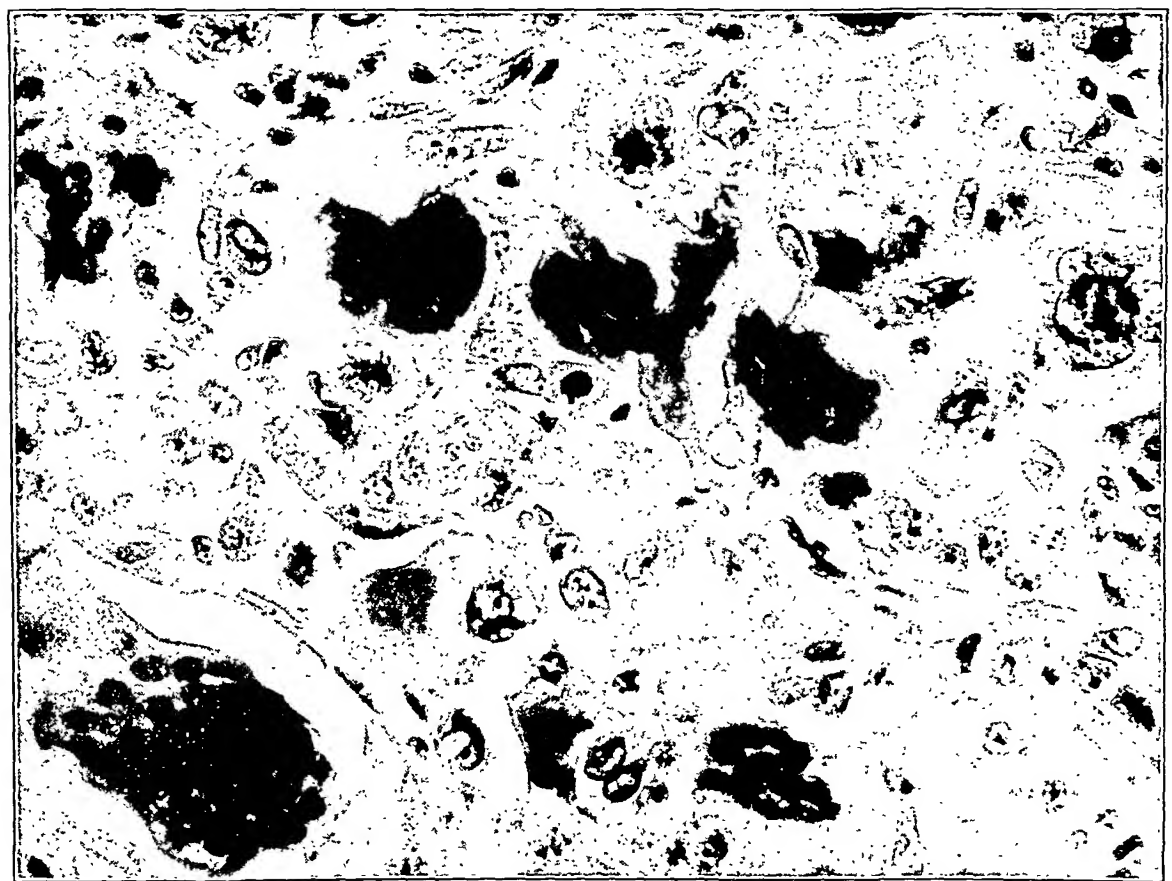
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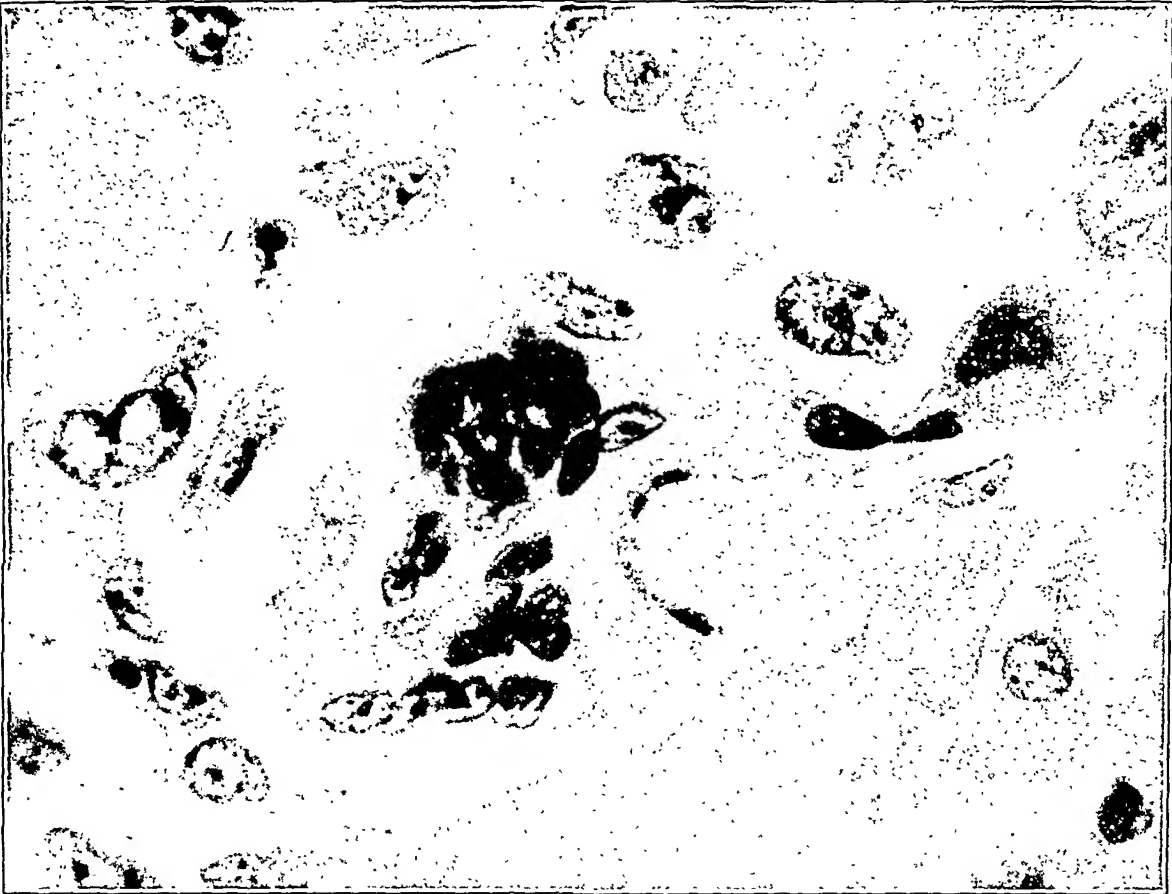
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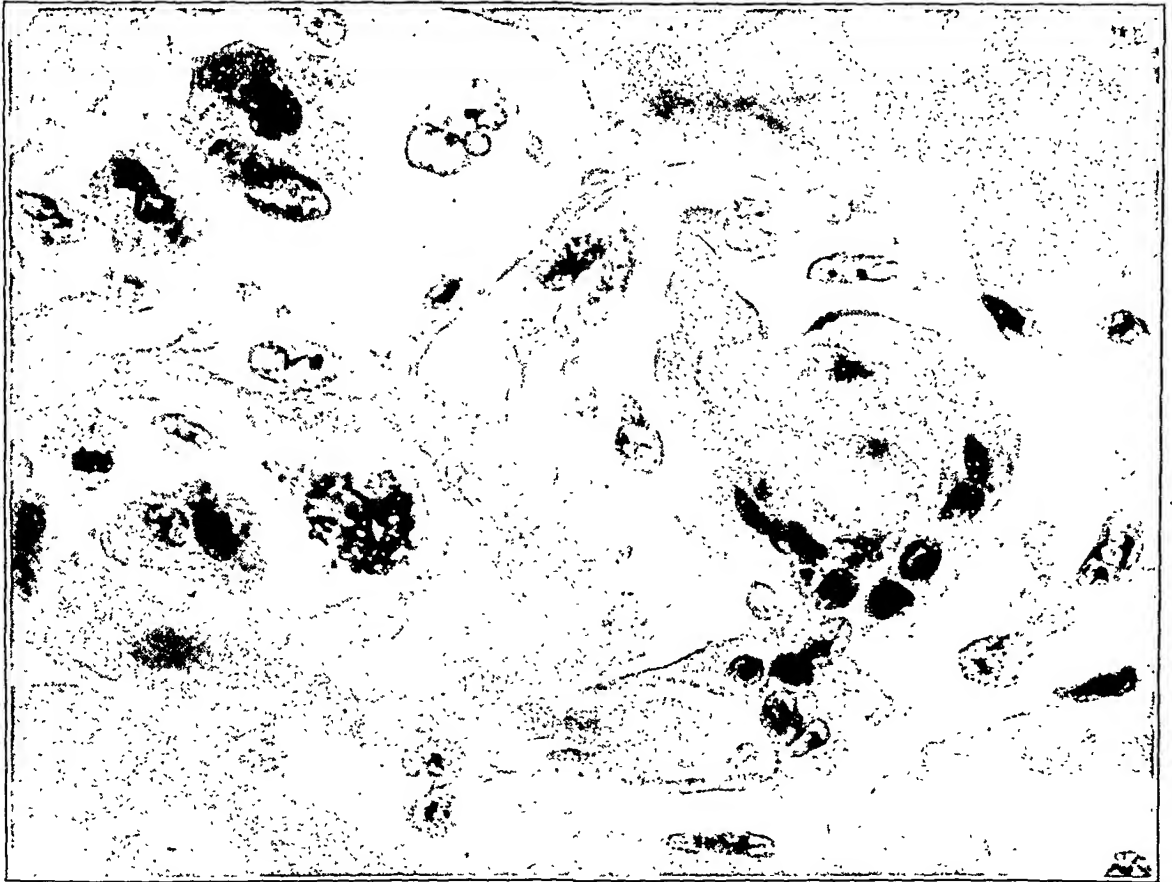
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times only one or two lobules are involved but as a rule the entire tuft is affected. The process is distributed uniformly throughout the whole kidney indicating that it is of toxic origin, not in foci as would be the case if it were due to the immediate presence of an infectious agent.

Counting the nuclei in sections of similar thickness and running through the middle of glomerular tufts showed two to three times as many in the affected glomeruli as in normal control ones. In addition to the glomerular lesion more or less degeneration of the renal epithelium was found, namely, hyaline droplet formation, necrosis and sometimes calcification. The tubules contained casts and occasionally showed regenerating epithelium.

We can offer no suggestion as to the cause of this glomerular lesion. During the course of our later experimental work, while studying kidneys under the oil immersion lens, we several times ran across mitoses in the capillary endothelium of the glomeruli. They may represent the early stage of this type of lesion but were evidently not due to the action of zinc because we found them occasionally in other rabbits also.

The type of lesion described here is entirely different from the one which is so often present in rabbits' kidneys and which leads to scarring (pitting) of the cortex. The irregular distribution of the latter would seem to indicate that it is of infectious origin.

Conclusion. Acute intracapillary glomerulonephritis sometimes occurs spontaneously in the rabbit.

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DESCRIPTION OF PLATE

PLATE 19

FIG. 1. Normal glomerular tuft in kidney of rabbit. $\times 500$.

FIG. 2. Mitosis in endothelial cell lining capillary of tuft in early stage of lesion. $\times 1000$.

FIG. 3. Diaster in tuft in well developed lesion. $\times 1000$.

FIGS. 4, 5 and 6. Well marked lesions showing occlusion of the capillaries owing to proliferation of the endothelial cells, enlargement of some of the lobules and marked increase in the number of nuclei as compared with the normal tuft in Fig. 1. $\times 500$.

SPONTANEOUS INTRACAPILLARY GLOMERULONEPHRITIS IN THE RABBIT *

F. B. MALLORY, M.D., AND FREDERIC PARKER, Jr., M.D.

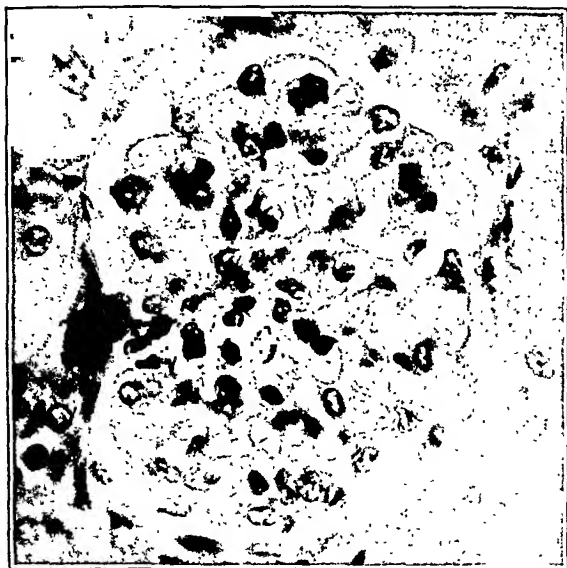
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At the twenty-fifth annual meeting of the American Association of Pathologists and Bacteriologists held in Washington, D. C., May 5 and 6, 1925, we reported¹ the presence of acute intracapillary glomerulonephritis as occurring in four out of eleven rabbits following the subcutaneous injection of 0.5 to 1 gm. of metallic zinc in fine powder form. The rabbits had been bred in the laboratory from good stock, there had been no epidemic of any sort so far as we were aware and no change in the character of the food. Moreover, the kidneys from several hundred rabbits had been examined in the course of other experimental work extending over a number of years and no similar lesion had ever been recognized. There is also a wide spread feeling among laboratory men that this type of lesion does not occur spontaneously. We, therefore, accepted our experiments as reliable and reported them accordingly.

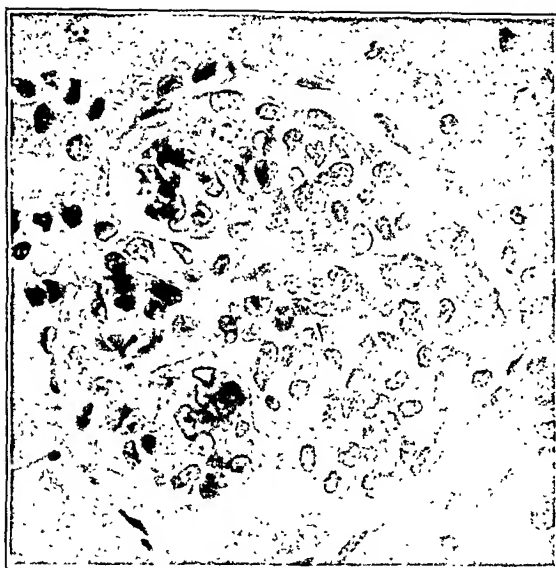
For over a year since then we have carried on a series of experiments with zinc, employing not only several of its salts but repeating the original experiment of injecting the metal in fine powder form subcutaneously, both in larger doses than before and over a much longer period of time. The metal dissolves more or less readily in the tissues owing to the lytic action of the leucocytes attracted to it and causes a slight local reaction but produces no significant effect on the kidney. We, therefore, publish this acknowledgment of our error in hopes that it may save some one else from drawing a similar wrong conclusion.

The type of lesion which occurred deserves to be put on record although only the acute stage of it has been observed. The process starts with proliferation of the lining endothelium in the capillaries in one or more of the lobules of the glomerular tuft. Mitotic figures are fairly numerous. Sometimes two can be found in a section of a single glomerulus. The proliferation of the endothelium leads to occlusion of the capillaries and enlargement of the lobule. Some-

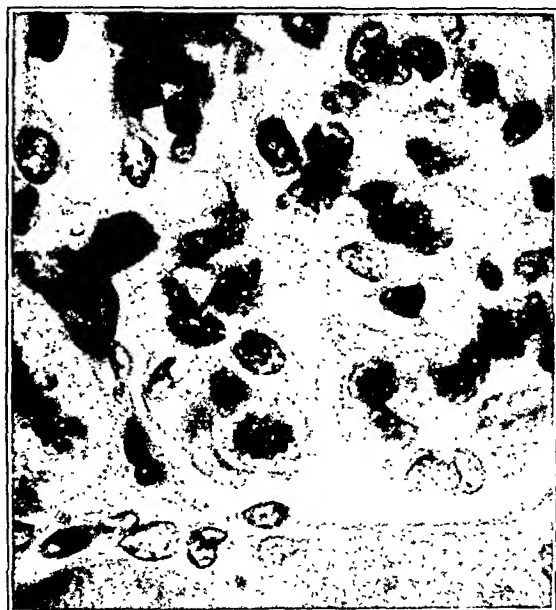
* Received for publication November 1, 1926.



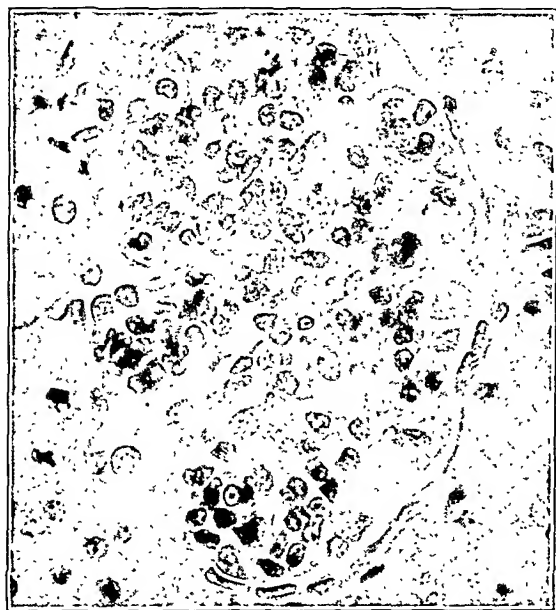
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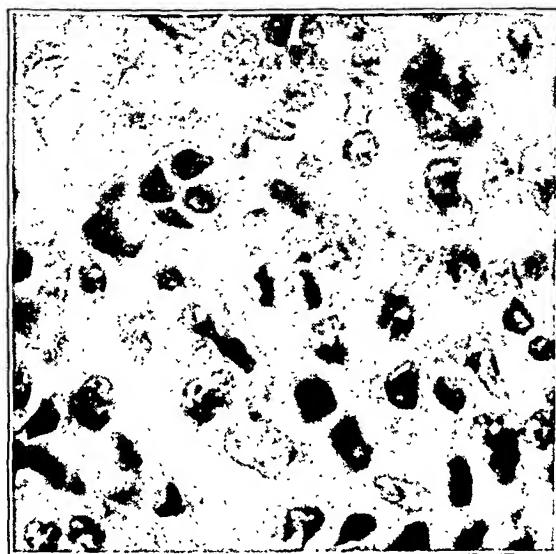
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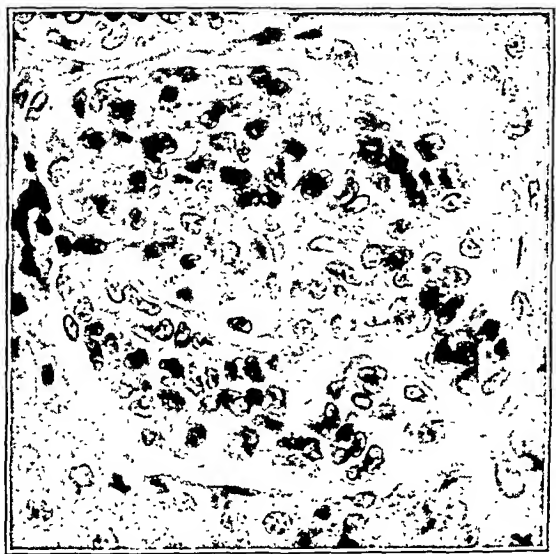
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description of the venous circulation of the uterus, based on the study of uteri in which the veins had been injected, were published in the year 1918 in an article ¹ dealing with the escape of foreign material into the venous circulation of the uterus. It then seemed to me that bits of the uterine mucosa, occasionally, might escape into the venous circulation during menstruation. I have frequently asked pathologists if they have ever found endometrial tissue in the lungs of women but have not ascertained that it has ever been observed.

As a result of these studies it is natural that I should consider the menstrual changes in the uterine mucosa a means of the dissemination of bits of endometrial tissue into the uterine circulation and as a possible source of certain instances of misplaced endometrial tissue in that organ and outside of it. To me it was a most plausible theory but lacked proof. I believed, however, that it must occur and could be demonstrated. It was just a question of a better knowledge of the venous and lymphatic circulations of the uterus and the histologic study of many sections of uteri, especially of those removed during menstruation.

THE INTRINSIC BLOOD SUPPLY OF THE UTERUS

The blood supply of the uterus was studied by injecting the vessels with melted gelatin containing bismuth subcarbonate and hardening the specimen in formalin. Stereoscopic roentgenograms were made of the entire uterus and also of cross slices of the same. The fine branches were studied in microscopic sections.

The general plan of the distribution of the intrinsic arteries of the uterus is as follows. Branches arise in pairs from each uterine artery and opposite each other, one branch penetrating the anterior and the other the posterior uterine wall. These two pairs of branches, which may be called arcuate arteries on account of their course in the myometrium, divide the uterine wall into a narrow outer or peripheral zone nourished by the peripheral branches of these arteries and a wide, inner or radial zone supplied by their radial branches (so named on account of their course). The latter terminate in the endometrium. The greater portion of the arterial supply of the uterus is directed toward its mucosa (Fig. 1). Each pair of arcuate arteries supplies a segment of the uterus corresponding to a segment of the Müllerian duct of that side.

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METASTATIC OR EMBOLIC ENDOMETRIOSIS, DUE TO THE MENSTRUAL DISSEMINATION OF ENDOMETRIAL TISSUE INTO THE VENOUS CIRCULATION*

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Over twelve years ago the writer began a series of experiments to determine the shape of the uterine cavity in normal and pathologic conditions. The technic was as follows. The uterus removed at operation or necropsy was placed in a basin of warm water and then filled with melted gelatin (about 15 per cent) containing in suspension bismuth subcarbonate or barium sulphate. This was introduced through the cervical canal by means of a syringe. After filling the uterine cavity the syringe was withdrawn, the cervix clamped in order to prevent the escape of the injection mass and the specimen placed in cold water until the gelatin had solidified. Stereoscopic roentgenograms of the uterus enabled one to obtain a clear picture of the form of the uterine cavity under various conditions and also of the lumina of the tubes if the latter were patent. In February, 1916, I removed a myomatous uterus from a patient who was menstruating at the time of the operation. On filling the uterine cavity with the injection mass I was surprised to find that it escaped from the severed uterine and ovarian veins. This was the first time that I had noticed this phenomenon. The following experiments were made. Uteri were curetted after their removal and the uterine cavity was filled with the mass. In many instances the injection mass escaped into the venous sinuses of the uterine wall and through the uterine and ovarian veins. These observations together with a

* Received for publication December 19, 1926.

large) and furthermore that this tissue, in its invasion of the myometrium, often extends in the spaces occupied by the vessels and sinuses of the uterine wall but is separated from the lumen of the latter by the endothelial lining of the vessel (Figs. 14, 15, 16, 17 and 18), as has been emphasized by Robert Meyer in his description of the relation of this ectopic endometrial tissue to the lymphatics of the uterine wall. (I believe, however, that the majority of these vessels are veins and not lymphatics.) It might be assumed that in the menstrual reaction of this misplaced endometrial tissue, bits of it might escape into its venous capillaries and also possibly into the lumen of a sinus of the uterine wall along which the endometrial tissue often extends in an extra- or retro-endothelial course. I also believe that the same applies to misplaced endometrial tissue of other origin than from a direct invasion of the uterine wall by its mucosa (Fig. 13). In one instance of an endometriosis of the cul-de-sac presenting in the posterior vaginal vault (a so-called adenomyoma of the recto-vaginal septum) the actual escape of the menstrual contents of two ectopic endometrial cavities into adjacent veins was found (Figs. 60 and 61) and, furthermore, bits of endometrium were present lying free in the lumina of and implanted on the lining of veins about these cavities (Figs. 58, 59 and 66). As a result of this finding I believe that a similar condition occasionally must arise in a direct endometriosis of the uterine wall. I have not definitely demonstrated it after a careful study of many sections from many blocks of tissue from uteri with a direct endometriosis, which were removed during the menstrual period, but believe that either it has been seen by others or will be found.

As bits of the uterine mucosa at times escape into the venous circulation of the uterus during menstruation, certain questions naturally arise. What is its pathologic and clinical significance? Could it possibly give rise to ectopic endometrial tissue not only in the uterine wall but also outside of that organ?

THE LYMPHATIC CIRCULATION OF THE UTERUS

Is it possible for bits of the uterine mucosa to be disseminated through the lymph vessels during menstruation, or may it actually invade these vessels and pieces of it escape through these channels? I have attempted to inject the lymphatics of the uterine wall and its mucosa but failed. Vessels which I have previously considered

The venous blood is conveyed from the uterine tissues mainly by the arcuate veins (corresponding in their general course with that of the arcuate arteries) which empty into the uterine plexus of veins, situated between the layers of the broad ligament. The arcuate veins receive blood from both the peripheral and the radial zones of the uterus (Fig. 2). The venous capillaries of the mucosa, some of which are dilated forming sinuses (Fig. 3), empty into the venous sinuses of the radial zone of the myometrium and these in turn into the arcuate veins. Some of the former are relatively large and radiate from the endometrium (Fig. 10). I have named these sinuses radial or, better, receiving sinuses, as they receive the greater portion of the blood from the endometrium and are continuous with the dilated capillaries (sinuses) of the latter. It is obvious that foreign material gaining access to the mucosal sinuses might escape through the receiving sinuses into the deeper vessels of the uterine wall. As there are no valves in the sinuses and veins of the uterine wall, the various physiologic changes in the venous pressure in these vessels might readily force foreign material in them into any of the sinuses and veins of the uterine wall, including those of the peripheral zone (Fig. 11), as they are to one side of the main blood stream which passes through the radial zone into the arcuate veins and thence to the uterine veins between the layers of the broad ligament. Only the principal veins of the uterus have well defined walls; the greater number of them, including the receiving sinuses, are usually only endothelial-lined spaces between the muscle bundles. In the non-injected specimen the veins and venous sinuses are often empty or contain very little blood and are therefore readily taken for lymphatics.

At menstruation some of the capillaries of the mucosa rupture and blood escapes into the tissues of the latter (Figs. 6, 7 and 8). Often bits of the mucosa may be found lying free in this extravasated blood. It is natural to assume that at times some of these bits might escape into the lumen of a ruptured mucosal capillary or sinus and be carried through a receiving sinus into the deeper vessels of the uterine wall. This does occur as will be shown.

The histologic study of the ectopic endometrial tissue in a direct or primary endometriosis (so-called adenomyoma of mucosal origin) shows that this tissue contains venous capillaries (Fig. 12) similar to those of the mucosa lining the uterine cavity (possibly not so

of the vessel. I can confirm Robert Meyer's observations as to the extra-endothelial course of endometrial tissue accompanying the vessels of the uterine wall in a direct endometriosis, but believe that the latter are usually venous sinuses and not lymph vessels. I also believe that the menstrual reaction of the endometrial tissue alongside of a vessel might cause rupture of the endothelial lining of the vessel and permit bits of endometrial tissue to escape into its lumen, but have not been able definitely to prove it.

Due to my inability to recognize the lymphatics of the uterine mucosa and the deeper tissues of the myometrium I have been unable to determine the part they take in the dissemination of endometrial tissue.

For anatomic and physiologic reasons it seems to me that metastases of endometrial tissue from the uterine mucosa and also from ectopic endometrial foci are more apt to be of venous than of lymphatic distribution. Still, this is a minor point which in time will be settled.

ENDOMETRIAL TISSUE IN THE VENOUS SINUSES OF THE UTERINE WALL DUE TO ARTEFACTS

If bits of endometrial tissue sometimes escape into the venous circulation of the uterus during menstruation, they should occasionally be seen in the veins and venous sinuses of uteri removed during the menstrual period. The problem would seem to be a very easy one. If they were not found after a careful study of many sections from many menstruating uteri it probably does not occur and if bits of endometrial tissue are discovered lying free in the lumina of these vessels the problem is solved. I encountered certain technical difficulties. The veins and venous sinuses of the uterus removed by operation are often empty or contain very little blood. The surgeon usually clamps the uterine side of the ovarian and uterine vessels and ligates the distal portion cutting between the clamps and the ligatures. After the uterus has been removed and the clamps released from the vessels the greater portion of the blood within the uterus escapes from the severed vessels and might carry with it any foreign material present in that blood. If the uterus is incised before it is fixed more blood escapes from its tissues. I partially obviated this difficulty by doubly ligating the vessels and

lymph vessels have corresponded to the venous capillaries and sinuses of uteri in which the veins have been injected. The sub-peritoneal lymph vessels about the uterine cornua and between the layers of the broad ligament may often be easily recognized without injecting them; but when one attempts to inject the deeper lymphatics of the uterine wall the venous capillaries and sinuses often become filled with the injection medium and may readily be taken for lymph vessels because they have a structure similar to the latter.

In an article ² published in 1922, I suggested that endometrial tissue might metastasize through lymph vessels, because I had found an endometrial polyp projecting into the lumen of a lymph vessel situated between the layers of the broad ligament. This polyp had arisen from the invasion of the vessel by endometrial tissue outside of the vessel pushing the endothelial lining of the lymphatic ahead of it. I also added that metastases might arise from the direct invasion of the uterine wall by the mucosa lining its cavity and from a similar invasion of the tubal wall by its mucosa.

In 1924 Halban published a preliminary communication ³ on the metastatic origin of misplaced endometrial tissue through the lymphatics. He believed that in the invasion of the myometrium by its mucosa some of the epithelium escapes into the lymph spaces between the muscle bundles of the uterine wall and is carried through these to the superficial lymphatics beneath the serosa and from there spreads by the lymph channels to other pelvic structures including the inguinal lymph nodes.

The following year I ⁴ discussed the possibility of metastasis of endometrial tissue through the lymph channels and suggested that the menstrual reaction of endometrial tissue encroaching upon or protruding into a lymphatic might disseminate bits of this tissue into its lumen and lead to metastasis. Robert Meyer, in a letter to me, justly criticised my suggestions and theories because I had not actually proved them. He referred to his own publications ⁵ and those of Kitai ⁶ where they had attempted to find evidence that endometrial tissue actually broke through the endothelial lining of the lymph vessels but had been unable to prove that it had. He well describes ⁷ the relation between the invading endometrial tubules and the lymph vessels and the way the former often follow these vessels and distort them without actually gaining access to the lumen

sinuses of many uteri removed at other times than during menstruation, and embolus-like lesions of endometrial tissue were found in only one uterus (see Case 2).

The histologic findings in four cases are reported, demonstrating the possible significance of the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus.

CASE 1. Patient aged 32, single. Uterus and right tube and ovary removed March 21, 1925, for a large submucous myoma on the second day of the menstrual period. Pieces of uterine mucosa were found in the blood of a mucosal sinus (Fig. 22), in a receiving sinus of the myometrium (Fig. 24), and also in other veins of the uterine wall (Fig. 25). A mural thrombus containing similar fragments was present in one of these veins (Fig. 26). The "endometrial tissue" in this thrombus stained poorly as compared not only with the uterine mucosa but also with the pieces of the latter found in the lumina of the other vessels of the same specimen (Figs. 27*a* and 27*b*), thus suggesting that they were undergoing degenerative changes and had been separated from the uterine mucosa for a longer time than the latter. There was associated an endometrial cyst of the right ovary which was fused with the posterior layer of the broad ligament, apparently resulting from a previous rupture or perforation of the cyst. The endometrial lining of the cyst showed the same menstrual reaction as that of the uterine cavity. Multiple lesions containing endometrial tissue involved the peritoneum about the right ovary and also were present in the posterior cul-de-sac. The distribution of these lesions was such as to indicate their origin from the escape of the contents of the endometrial cyst of the ovary into the peritoneal cavity.

CASE 2. Patient aged 33, single. Uterus and both tubes and ovaries removed Nov. 20, 1924, three weeks after the last menstrual period, for an extensive peritoneal endometriosis associated with bilateral ovarian cysts of endometrial type. The posterior wall of the uterus was deeply invaded by endometrial tissue apparently developing on its peritoneal surface or at least in the peripheral zone of the uterus. Endometrial emboli were found in four veins of the uterine wall, all of them either arcuate veins or their peripheral branches. The histologic relation of these emboli to the contents of the veins and their lining was such as to lead one to believe that they could not be artefacts (Figs. 28 and 29). Most of the endometrial

cutting between the ligatures before removing the uterus. The entire specimen was hardened in formalin and blocks were not cut from it until it was fully fixed. The uterus was cut into cross slices and these slices were cut in halves or quarters depending upon their size and embedded in celloidin. The majority of the veins and venous sinuses of the uterine wall were still found to be empty or to contain very little blood, but the uterine plexus of veins on either side of the uterus was distended with blood. During fixation the uterus evidently contracts and forces the greater portion of the blood in its sinuses into the veins on either side of it.

Bits of uterine mucosa were found in the veins and sinuses of the wall of menstruating uteri. A careful study of these sections and the process of embedding showed that some of these findings were due to artefacts. The menstruating uterine mucosa is very friable; bits of it break off during the process of embedding and readily drop into the empty veins and sinuses of the uterine wall. These appear as endometrial emboli in the stained sections (see Figs. 19, 20 and 21). If, however, pieces of the uterine mucosa are found surrounded by blood in a vein or sinus or attached to the wall of the vessel by fibrin, it is evident that they reached this situation before the tissues were fixed. It is also possible that bits of the uterine mucosa may be carried into the venous sinuses of the uterine wall in cutting blocks from the unfixed uterus.

ENDOMETRIAL TISSUE IN THE VEINS AND VENOUS SINUSES OF THE UTERINE WALL DUE TO THE MENSTRUAL DISSEMINATION OF THIS TISSUE INTO THESE VESSELS

Fragments of endometrial tissue were found either in the blood of the veins and venous sinuses of the uterine wall or attached to the lining of these vessels by fibrin in three uteri removed during the menstrual period (Cases 1, 3 and 4). In two other uteri, removed during the menstrual period, clumps of epithelium-like cells which I was unable to identify, were found in the blood or attached to the walls of veins. A careful study of three other uteri removed during menstruation failed to reveal any embolic endometrial tissue in the vessels of the uterine wall but several artefacts were present in sections from one uterus (see Figs. 19a, 20 and 21). Sections were carefully examined for endometrial tissue in the veins and venous

and other findings in the case strongly suggested this possibility (see Figs. 33, 34 and 35).

CASE 4. Patient aged 41, married; one child 14 years old. Uterus, both tubes and ovaries removed March 9, 1926, on the second day of the menstrual period, for a peritoneal endometriosis fusing the anterior surface of the uterus with the bladder, with partial obliteration of the anterior cul-de-sac; a similar lesion obliterating the bottom of the posterior cul-de-sac, with invasion of the sigmoid causing partial intestinal obstruction, and extension downward between the rectum and the vagina and into the posterior vaginal vault. Following the suggestion of Dr. William P. Graves⁸ a temporary colostomy was made which later closed spontaneously.

Ovaries were normal and tubes were patent. An endometriosis, in many ways displaying the histologic structure of the direct type, was present in the left half of the posterior uterine wall (Figs. 36, 38 and 39) with but slight invasion of the right half of the posterior uterine wall and that near the fundus. In the right half of the posterior uterine wall endometrial emboli were lying free in the lumina of some of the veins of the peripheral zone (Figs. 37, 43, 53 and 65). Multiple embolic or metastatic growths of endometrial tissue were found in the arcuate veins and also in the peripheral veins of that side (Figs. 37, 49 and 52). Serial sections showed that these deposits of endometrial tissue were attached to the walls or lining of these vessels and were not continuous with any endometrial tissue outside of the vessel. They were evidently due to a localized metaplasia of the endothelial lining of the vessels or an implantation (anchoring) of bits of endometrial tissue similar to those which were found floating about in the lumina of some of them. The study of the specimen suggested that they primarily arose from the menstrual dissemination of fragments of endometrial tissue from the uterine mucosa into receiving sinuses rather than from the menstrual reaction of the ectopic endometrial tissue in the left half of the posterior uterine wall. In only a very few areas of ectopic endometrial tissue of the left half of the posterior uterine wall was there any suggestion of a menstrual reaction (Fig. 40). Had these emboli arisen from this source, one would have expected to have found more of them in the sinuses and veins of that half of the uterus rather than in those of the opposite side. As it was, only a very few were found in the left half of the posterior uterine wall and those in the peripheral zone

tissue in these vessels, both epithelium and stroma, stained poorly as compared with the mucosa lining the uterine cavity, suggesting degenerative changes. I believe that they might have escaped into the venous circulation of the uterus during menstruation. The last flow occurred three weeks before the operation.

CASE 3. Patient aged 53, single. Uterus and both tubes and ovaries removed April 10, 1926, for an endometriosis of its anterior wall, apparently of the direct type (Figs. 14, 15, 16, 17 and 18). Patient had been flowing for five weeks prior to the operation. A gland-like arrangement of "uterine epithelium" was found attached by fibrin to the wall of a receiving sinus (Figs. 32 and 34), thus demonstrating that it must have reached this situation before the tissues had been fixed. In another receiving sinus a polypoid growth of endometrial tissue, apparently covered by endothelium and consisting of a typical uterine gland surrounded by stroma, was attached by a slender pedicle to its wall (Figs. 30, 31 and 33). While an endometriosis of the uterine wall involved the tissues about a branch of this sinus at another level of the block, serial sections showed that the gland of the polyp was not continuous with any endometrial tubule outside of the sinus. (In all cases where I have been able to obtain either serial sections or many sections of polypoid invaginations of endometrial tissues into vessels, it was possible to demonstrate that the apparent gland-like structure in the polyp was but a section of an epithelial tubule continuous with those outside of the vessel (see Figs. 14 to 18 inclusive).) The endometrial polyp in this case must have arisen either from (1) a metaplasia of the endothelial lining of the sinus, (2) an implantation of a fragment of endometrial tissue escaping into the sinus from the uterine mucosa during menstruation, (3) an implantation of a piece of endometrial tissue escaping into a branch of the sinus during the menstrual reaction of the heterotopic endometrial tissue about it, or (4) if it previously had been continuous with the endometrial tissue about a branch of the sinus (at another level), this connection in some way must have been severed.

Another interesting problem presents itself in this case and that is whether or not some of the ectopic endometrial tissue of the uterine wall may have been of metastatic or embolic origin rather than arising from the direct invasion of the myometrium by its mucosa. The uterine epithelium adherent to the wall of a sinus, the polyp

wall by its mucosa found and that apparently for only a short distance. Its continuation with the extensive endometrial lesions of that wall was not definitely established. The distribution of the endometrial tissue in this portion of the posterior uterine wall (Fig. 36) conformed with the distribution of the bismuth in corresponding sections of the walls of uteri in which the veins had been injected. The endometrial tissue in this lesion was nearly everywhere of the direct type, *i. e.*, wherever the lumen of a vein or sinus could be seen the endometrial tissue was retro- and not intra-endothelial, except in a few areas (where it possibly communicated with the lumen of a vein) such as were present in the peripheral zone. I believe that a metastatic lesion may possibly become entirely retro-endothelial by the growth of the endothelial lining of the vessel over it, as endothelium grows over a thrombus, and subsequently it may take a retro-endothelial course in its extension and thus be histologically indistinguishable from the lesion of a direct endometriosis. In several of the embolic lesions the endothelium of the vein or sinus had grown over portions of the endometrial implant (see Figs. 41, 50 and 52) so as to suggest that it might completely cover it, although nowhere was this conclusively shown.

The posterior surface of the body of the uterus was not adherent but the bottom of the posterior cul-de-sac was occluded by an extensive endometriosis fusing the posterior wall of the cervix with the recto-sigmoid. The nature of the intestinal lesion was not ascertained as the sigmoid was not resected.

The endometriosis of the portion of the posterior vaginal wall, which was excised, was as interesting as that of the uterus. Multiple ectopic endometrial cavities were found filled with blood in which were floating bits of endometrial tissue (Figs. 57, 58 and 60). Similar fragments were found lying free in the lumina of nearby veins and also anchored to or implanted on the walls of veins (Figs. 58, 59 and 66). The actual escape into a vein of the menstrual contents of two of these endometrial cavities could be demonstrated (Figs. 60 and 61). Here again we have evidence that endometrial tissue may be disseminated into veins during menstruation and actually become implanted on the walls of veins, thus demonstrating that the fragments of endometrium set free by the menstrual reaction are sometimes alive and capable of becoming implanted under favorable conditions.

(Fig. 41), while many were found in the veins and venous sinuses of the opposite side. A bit of endometrial tissue was found in a sinus of the uterine mucosa (Fig. 55) and also embolic lesions were found in the receiving sinuses of the right half of the posterior uterine wall (Figs. 53 and 54), thus suggesting that it was through such channels that the endometrial tissue had primarily escaped in order to reach the arcuate and peripheral veins of the side in which the embolic lesions were most numerous. It seems reasonable to believe that these endometrial vegetations in the sinuses and veins arose from the anchoring or implantation of endometrial fragments similar to those found floating in these vessels and were primarily derived from the menstrual dissemination of endometrial tissue from the uterine mucosa into the venous circulation of the uterus. On the other hand, some of the endometrial tissue floating in these vessels might be pieces cast off by a menstrual reaction in the embolic vegetations of endometrium growing on their walls.

A peritoneal endometriosis was present in the anterior cul-de-sac fusing the anterior surface of the uterus with the anterior layer of the broad ligament and the peritoneum covering the bladder and apparently arising by peritoneal implantation (Fig. 56). Only the peripheral zone of the anterior uterine wall was invaded by this tissue and no deeper than the corresponding invasion of the anterior layer of the broad ligament and the peritoneum covering the bladder. The entire uterine wall was cut into blocks and many sections were studied from each block. Endometrial tissue was not found in the deeper portions of the anterior uterine wall. Could the endometriosis in this situation possibly have arisen from metastasis to the peripheral vessels of the anterior uterine wall, similar to that already described, and later extending to the peritoneal surface? Such an origin cannot be excluded. Had it been metastatic through veins or lymphatics we would expect to find in it emboli similar to those of the right half of the posterior uterine wall but none was found. In a few places, however, a possible communication of the endometrial tissue with the lumen of a sinus was found where portions of the endometrial tissue were definitely intra- and not retro-endothelial, thus suggesting a possible metastatic origin. It is also possible that the extensive endometriosis of the radial zone of the left half of the posterior uterine wall was of metastatic rather than of direct origin as in only one place was an invasion of the uterine

some of those in the vagina, vulva, groin, or even the umbilicus, the latter through the round ligament and epigastric veins.

If menstrual blood carrying with it bits of endometrial tissue escapes into the venous circulation of the uterus and these bits sometimes become implanted on the endothelial surface of the veins and venous sinuses of the uterine wall, and if a like condition arises from the menstrual reaction of the mucosa of ectopic endometrial cavities in the vagina, we might infer that bits of endometrial tissue carried by menstrual blood escaping into the peritoneal cavity from any source (such as a back flow through the tubes from the uterine cavity, from the rupture or perforation of an endometrial cyst of the ovary or the menstrual reaction of endometrial tissue on the surface of the various pelvic structures) might become implanted on the mesothelial surface of the peritoneum and give rise to at least some of the lesions of peritoneal endometriosis. Jacobson has shown by his experimental work in rabbits and monkeys that bits of the uterine mucosa of these animals, scattered in their peritoneal cavities, become implanted on the peritoneum causing a peritoneal endometriosis similar to that found in human beings. The findings in the cases just reported demonstrate that bits of endometrial tissue disseminated by menstruation are sometimes alive and can become implanted on the endothelial lining of veins and venous sinuses. We know that menstrual blood, containing bits of endometrium, at times escapes into the peritoneal cavity from the above mentioned sources. Peritoneal endometriosis in women often occurs in situations and under conditions indicating (or at least suggesting) its origin from these sources.

THE ORIGIN OF ENDOMETRIAL TISSUE IN UTERINE ENDOMETRIOSIS

The study of endometriosis of the uterine wall demonstrates that it may arise in four, and possibly more, ways.

1. The direct invasion of the uterine wall by its mucosa or by tubal mucosa — a direct or primary uterine endometriosis.
2. The invasion of the external portion of the uterine wall by the direct extension of endometrial tissue from an ectopic endometrial focus in the pelvis — an indirect or secondary uterine endometriosis, by extension.

What was the origin of the ectopic endometrial tissue in this case? I have proved that some of the lesions were of metastatic or embolic origin and also was able to find a direct invasion of the uterine wall by its mucosa. It was not possible, however, to determine whether or not the latter gave rise to all of the endometrial tissue in the extensive endometriosis of that portion of the uterine wall. It was also shown that there were peritoneal lesions of implantation type, possibly due to the escape of menstrual blood through the tubes into the peritoneal cavity. The tubes were patent and endometrial tissue was not found in the ovaries. If one method for the extension or dissemination of endometrial tissue was primarily responsible for the endometriosis in this case, we must choose the metastatic (embolic), *i. e.*, the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus. Some of the tissue emboli might have become retro-endothelial and in their subsequent extension caused the endometriosis of the radial zone of the left half of the posterior uterine wall in which the distribution of endometrial tissue simulated that of the bismuth in the uterine wall whose veins had been injected and were of the type of a direct endometriosis. Metastases in the subperitoneal veins of the uterine walls might have subsequently extended through to the peritoneal surface causing a peritoneal endometriosis with a later extension to the peritoneum covering the bladder in the anterior cul-de-sac and the sigmoid and recto-vaginal septum in the posterior cul-de-sac. I admit the possibility of the above but am inclined to believe that there was more than one method of origin of the ectopic endometrial tissue in this case.

THE BEARING OF THESE STUDIES ON THE ETIOLOGY OF OTHER VARIETIES OF ENDOMETRIOSIS THAN THOSE JUST DESCRIBED

If fragments of endometrial tissue escape into the venous circulation of the uterine wall during menstruation and become implanted on the endothelial surface of the veins and venous sinuses of that organ and if a similar condition arises in veins about misplaced endometrial cavities in the vagina, it is natural to assume that a like implantation of this tissue might occur in veins remote from these endometrial cavities. This might account for the origin of misplaced endometrial tissue at a distance from the uterus, such as

By serial sections it was shown that these growths either arose from or were implanted on the walls or linings of these vessels and did not arise from the invasion of the latter by endometrial tissue from without. These embolus-like growths of endometrial tissue must have originated either from a localized metaplasia of the endothelial lining of the veins and venous sinuses or else from the actual anchoring and implantation of endometrial tissue similar to that found free in some of the vessels of the specimen. The study of the entire uterus demonstrated that, while some of the endometrial emboli lying free in the vessels of the uterine wall might have arisen from the menstruation of ectopic endometrial tissue in that organ, the latter primarily were derived from the mucosa lining the uterine cavity. In the endometriosis of the posterior vaginal wall of this case, similar endometrial emboli and embolic vegetations of endometrial tissue were present there in veins about misplaced endometrial cavities and the actual escape of the menstrual contents of two of these cavities into a vein was seen.

In a second uterus, also removed while the patient was flowing (Case 3), somewhat similar lesions were found in which their embolic origin was not as definitely established as in Case 4. Nevertheless I believe that they had a similar origin.

If these observations are correctly interpreted, they show that bits of endometrial tissue disseminated by menstruation from the mucosa lining the uterine cavity and also from ectopic endometrial foci, are not always dead but are sometimes alive and are capable of becoming implanted on the endothelial surface of nearby veins and venous sinuses.

They further suggest that bits of endometrial tissue carried by menstrual blood into the venous circulation might cause metastatic growths of endometrial tissue at a distance from the original focus, and also that similar fragments of endometrial tissue carried by menstrual blood escaping from any source into the peritoneal cavity at times might cause the lesions of peritoneal endometriosis.

CONCLUSIONS

1. Fragments of endometrial tissue, at times, are disseminated into the venous circulation during menstruation, from the mucosa lining the uterine cavity and also from ectopic endometrial foci.

3. From endometrial tissue implanted or developing on its peritoneal surface — an implantation or peritoneal uterine endometriosis.
4. From the menstrual dissemination of endometrial tissue into the venous circulation of the uterus, either from the mucosa lining its cavity or from ectopic endometrial tissue in the myometrium — an embolic or metastatic uterine endometriosis.
5. The possibility of metastasis through the lymphatics, and also of developmental inclusions of the uterine mucosa in the myometrium, must be considered. The origin of endometrial tissue from a metaplasia of the endothelial lining of vessels does not appeal to me.

SUMMARY

A histologic study was made of sections of uteri removed during the various stages of the menstrual cycle, in which the veins had been injected with bismuth. By this means it was demonstrated that there are venous capillaries and large venous sinuses in the uterine mucosa and that the latter empty into similar sinuses (receiving) in the uterine wall. During menstruation, blood escapes from the mucosal vessels into the surrounding tissues, and bits of the mucosa are often set free in the extravasated blood. These studies suggest that this menstrual blood containing fragments of endometrial tissue, at times escapes through a ruptured mucosal sinus into the venous circulation of the uterus.

Sections of misplaced endometrial tissue, wherever situated and irrespective of its origin, also suggest that a like dissemination of fragments of this tissue occurs during menstruation.

In menstruating uteri bits of the uterine mucosa at times actually escape into the venous circulation of the uterus through these channels. I have not been able definitely to prove this in a direct endometriosis of the uterine wall but believe that it also must occur in this condition. The escape into veins of the contents of two ectopic endometrial cavities was found in an endometriosis of the posterior vaginal wall.

In one uterus removed during menstruation (Case 4) in which bits of endometrial tissue were found in the blood in veins and venous sinuses of the uterine wall, multiple embolic or metastatic-like growths of endometrial tissue also were present in these vessels.

PLATE 20

FIG. 1. The general plan of the distribution of the intrinsic uterine arteries as seen in a cross-section of the body of the uterus. Composite tracing (slightly enlarged) of roentgenograms of thin cross slices of the uterus; arteries injected with bismuth. The arcuate arteries, which arise in pairs from each uterine artery, divide the uterine wall into a narrow outer or peripheral zone nourished by the peripheral branches of these arteries and a wide inner or radial zone supplied by their radial branches. The latter terminate in the mucosa. The greater portion of the arterial supply of the uterus is directed toward its mucosa.

FIG. 2. The general plan of the venous outlets of the uterine tissues as seen in a cross-section of the body of the uterus. Tracing (enlarged and with some of the receiving sinuses accentuated) of a roentgenogram of a thin cross slice of the uterus, veins injected with bismuth; hyperemia due to tubal pregnancy. The venous blood is conveyed from the uterine tissues mainly by the arcuate veins which empty into the uterine plexus of veins situated between the layers of the broad ligament. The arcuate veins receive blood from both the peripheral and the radial zones of the uterus. The venous capillaries of the mucosa, some of which are dilated forming sinuses (Fig. 3), empty into the venous sinuses of the radial zone of the myometrium and these in turn into the arcuate veins. Some of these sinuses are relatively large and radiate from the endometrium (R of illustration). It is obvious that foreign material gaining access to the lumina of the mucosal sinuses might escape through the radial or receiving sinuses (R) into the deeper sinuses and veins of the uterine wall.

2. Metastatic or embolic endometriosis arises from the implantation of these emboli in nearby veins.

3. Endometrial tissue set free by menstruation, therefore, is sometimes not only alive but may actually continue to grow if transferred to situations favorable to its existence.

The colored illustrations for this paper were made by Mrs. M. R. Marden and the photomicrographs by Mr. James A. Glenn.

The demonstration of the origin and course of the ectopic endometrial tissue in these specimens was made possible by the technical skill and care of Miss Isabel Peck.

These I thank for their interest and coöperation.

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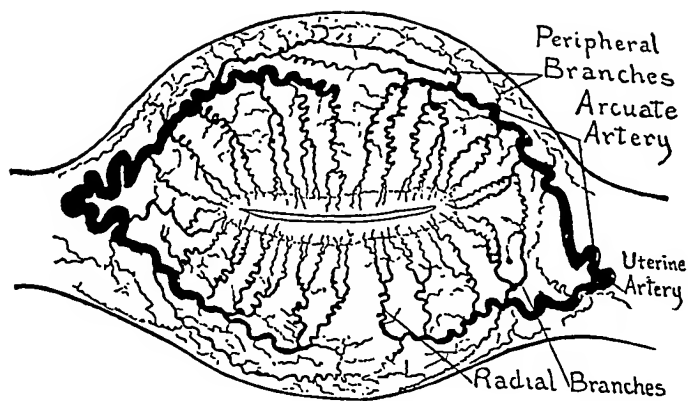
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PLATE 21

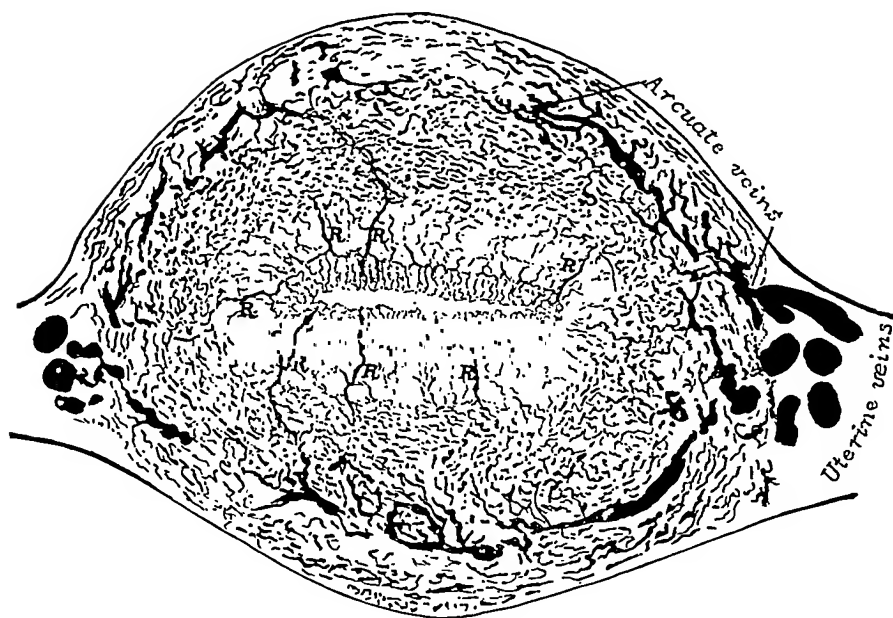
FIG. 3. Photomicrograph ($\times 25$) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; from a patient with tubal pregnancy. Condition present corresponds with that found at the close of the menstrual period. Two receiving sinuses are shown, one of which extends almost to the surface of the uterine mucosa. The portion of the sinus situated within the mucosa might be designated a mucosal sinus. These sinuses are but spaces lined by endothelium and without definite walls. In the non-injected specimen they are often empty or contain very little blood and therefore could readily be mistaken for lymph vessels. Any foreign material in the lumen of the mucosal sinus might easily escape into the receiving sinus of the muscularis with which it is continuous.

FIG. 4. Photomicrograph ($\times 25$) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; interval stage of the menstrual cycle. The mucosa is about twice as thick as that shown in Fig. 3. A receiving sinus is present and due to the postmenstrual growth of the mucosa, the sinus is more deeply situated than those shown in Fig. 3.

FIG. 5. Photomicrograph ($\times 10$) of a section of the uterine mucosa and underlying muscularis in the angle between the anterior and posterior uterine wall, veins injected with bismuth; premenstrual stage of the menstrual cycle. The mucosa is much thicker than that shown in Fig. 4 (less than half the magnification). Several dilated capillaries or mucosal sinuses are present. As the principal menstrual reaction usually occurs in the superficial portion of the mucosa, could bits of the latter escape into these sinuses during menstruation?



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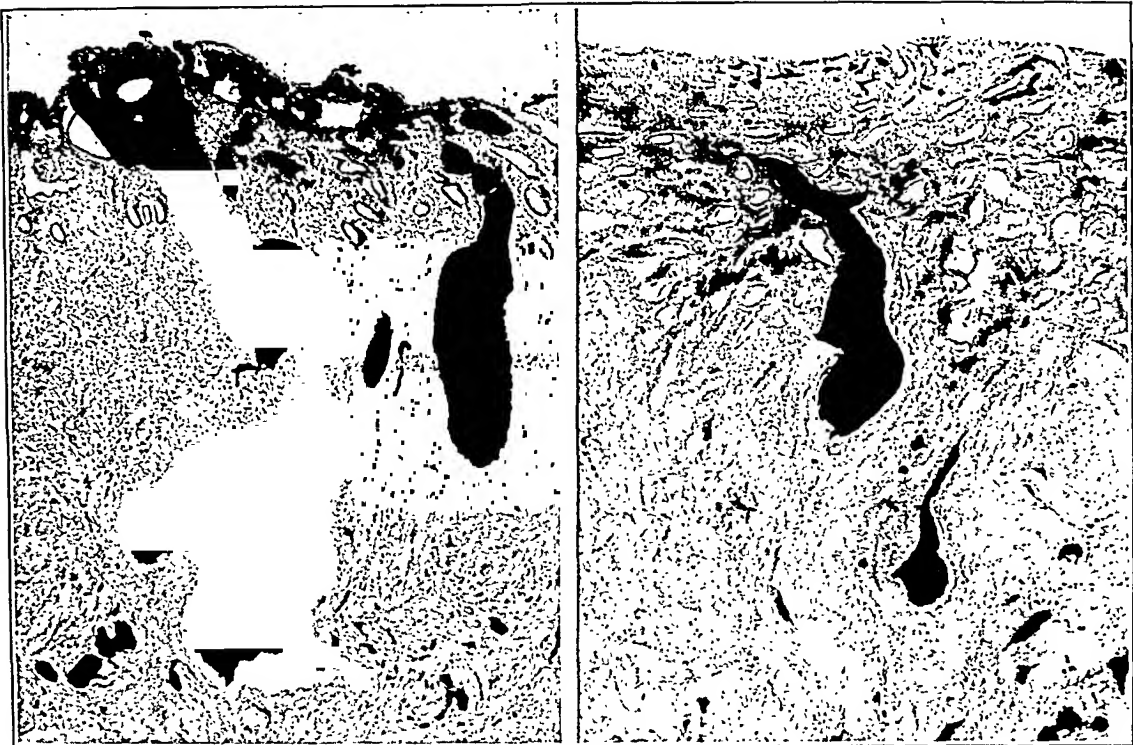


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FIGS. 6, 7 and 8. Three photomicrographs ($\times 20$) of sections of the uterine mucosa and underlying muscularis, veins injected with bismuth; patient menstruating. All sections from the same uterus but from different portions and showing different stages in the menstrual reaction. The photomicrograph to the left shows the dilated mucosal capillaries, the middle one the rupture of the same, due to the menstrual reaction, thus permitting the injection mass to escape into the tissues of the mucosa. Fig. 7 represents a still later stage of the menstrual reaction. The superficial layer of the uterine mucosa has been separated from the deeper layer by the extravasated injection mass and bits of the uterine mucosa lie free in this mass just as they are found in the extravasated blood of the non-injected menstruating endometrium. Could menstrual blood carrying with it bits of the mucosa escape into the ruptured dilated venous capillaries and from these into the venous circulation of the uterus?

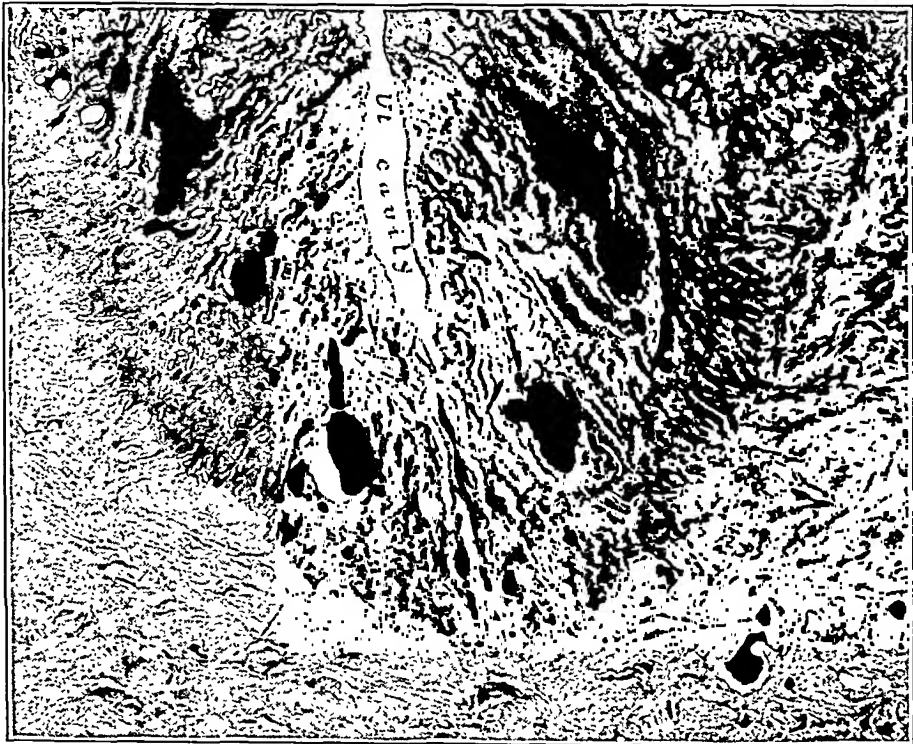
FIG. 9. Photomicrograph ($\times 25$) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; end of the first day of the menstrual period. A mucosal sinus is shown with rupture of its endothelial lining permitting the injection mass to escape into the tissues of the mucosa. It is conceivable that fragments of the uterine mucosa set free by the extravasated blood, might at times escape into the lumen of such a sinus and be carried with that blood into the venous circulation of the uterus (see Fig. 10).

FIG. 10. Photomicrograph ($\times 5$) of a cross-section of the uterine wall including its mucosa (section from which the photomicrograph shown in Fig. 9 was made). The mucosal sinus (M.S.) of the endometrium, which has ruptured, is shown and also a receiving sinus which carries the venous blood from the mucosa. (It is not evident that the mucosal sinus in this photomicrograph empties into this receiving sinus but either it does, or else into one like it.) Blood carrying with it any material, such as bits of the uterine mucosa, which had escaped into the lumen of a mucosal sinus, might readily be carried through a receiving sinus into any of the venous sinuses of the uterine wall and especially into the arcuate veins as the main blood stream is from the mucosa through the receiving sinuses into these veins.



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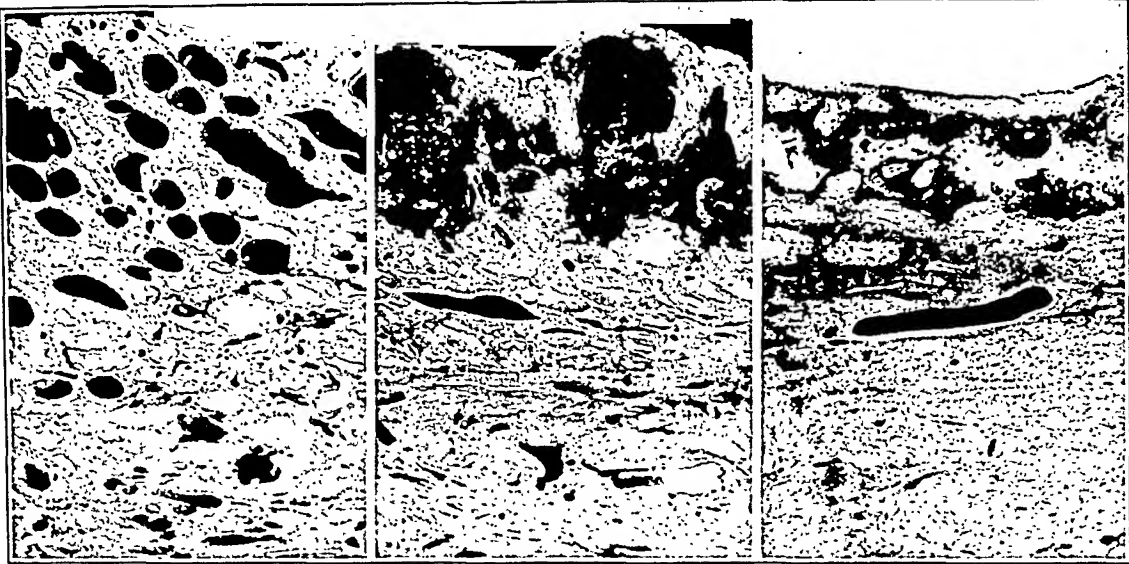


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FIGS. 6, 7 and 8. Three photomicrographs ($\times 20$) of sections of the uterine mucosa and underlying muscularis, veins injected with bismuth; patient menstruating. All sections from the same uterus but from different portions and showing different stages in the menstrual reaction. The photomicrograph to the left shows the dilated mucosal capillaries, the middle one the rupture of the same, due to the menstrual reaction, thus permitting the injection mass to escape into the tissues of the mucosa. Fig. 7 represents a still later stage of the menstrual reaction. The superficial layer of the uterine mucosa has been separated from the deeper layer by the extravasated injection mass and bits of the uterine mucosa lie free in this mass just as they are found in the extravasated blood of the non-injected menstruating endometrium. Could menstrual blood carrying with it bits of the mucosa escape into the ruptured dilated venous capillaries and from these into the venous circulation of the uterus?

FIG. 9. Photomicrograph ($\times 25$) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; end of the first day of the menstrual period. A mucosal sinus is shown with rupture of its endothelial lining permitting the injection mass to escape into the tissues of the mucosa. It is conceivable that fragments of the uterine mucosa set free by the extravasated blood, might at times escape into the lumen of such a sinus and be carried with that blood into the venous circulation of the uterus (see Fig. 10).

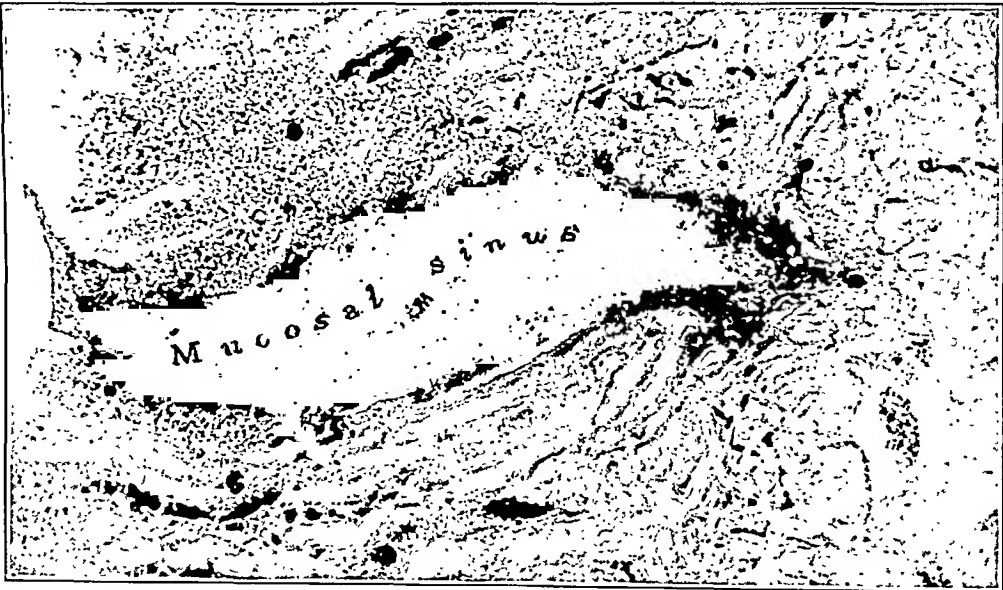
FIG. 10. Photomicrograph ($\times 5$) of a cross-section of the uterine wall including its mucosa (section from which the photomicrograph shown in Fig. 9 was made). The mucosal sinus (M.S.) of the endometrium, which has ruptured, is shown and also a receiving sinus which carries the venous blood from the mucosa. (It is not evident that the mucosal sinus in this photomicrograph empties into this receiving sinus but either it does, or else into one like it.) Blood carrying with it any material, such as bits of the uterine mucosa, which had escaped into the lumen of a mucosal sinus, might readily be carried through a receiving sinus into any of the venous sinuses of the uterine wall and especially into the arcuate veins as the main blood stream is from the mucosa through the receiving sinuses into these veins.



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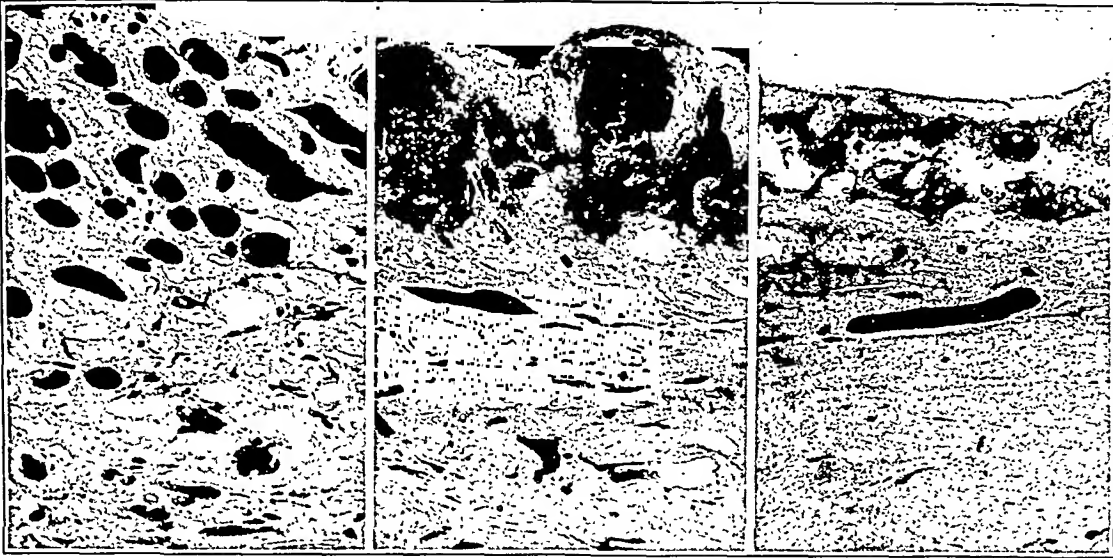


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FIG. 11. Photomicrograph ($\times 25$) of a cross-section of the peripheral zone of the uterine wall, veins injected with bismuth; premenstrual stage of the menstrual cycle. The veins and venous sinuses in this section are but spaces, between the muscle bundles, lined by endothelium. They empty into the arcuate veins, bottom of the photomicrograph. (Some of the injection mass has fallen out of the larger vessels.) In the non-injected specimen these vessels are often empty or contain very little blood and may readily be taken for lymphatics. There are no valves in the veins and venous sinuses of the uterine wall. The various physiologic changes in the venous pressure in these vessels, due to uterine relaxation and contraction, might force foreign material suspended in the blood into any of the veins and sinuses of the uterine wall including those of the peripheral zone, since they are to one side of the main blood stream from the mucosa to the arcuate veins, and thence to the uterine veins. For physiologic reasons we might expect to find bits of the uterine mucosa, escaping into the venous circulation, in the vessels of the peripheral zone, even in the small vessels near the serosa where they might be retained.

FIG. 12. Photomicrograph ($\times 25$) of a section of the uterine wall with a direct or primary endometriosis (adenomyoma due to the invasion of the uterine wall by its mucosa), veins injected with bismuth. Dilated venous capillaries (sinuses) are present in this misplaced uterine mucosa similar to those of the mucosa lining the uterine cavity. In the menstrual reaction of misplaced endometrial tissue, fragments of it are set free in extravasated blood just as they are set free in the extravasated blood of the menstruating mucosa lining the uterine cavity. Could some of these bits gain access to the lumina of its venous sinuses and be carried into the venous circulation of the uterine wall? I have examined many sections of primary endometriosis in uteri removed during menstruation, and have not been able to prove that this occurs. I believe that it must occur and will be definitely proved.

FIG. 13. Photomicrograph ($\times 25$) of a section of the uterine wall with an endometriosis near its serosa; veins injected with bismuth. The patient had bilateral ovarian hematomas of endometrial type associated with a peritoneal endometriosis. The misplaced endometrial tissue in this section also contains dilated venous capillaries or sinuses. In the menstrual reaction of this misplaced "uterine mucosa" bits of the latter might also gain access to the lumina of its sinuses.



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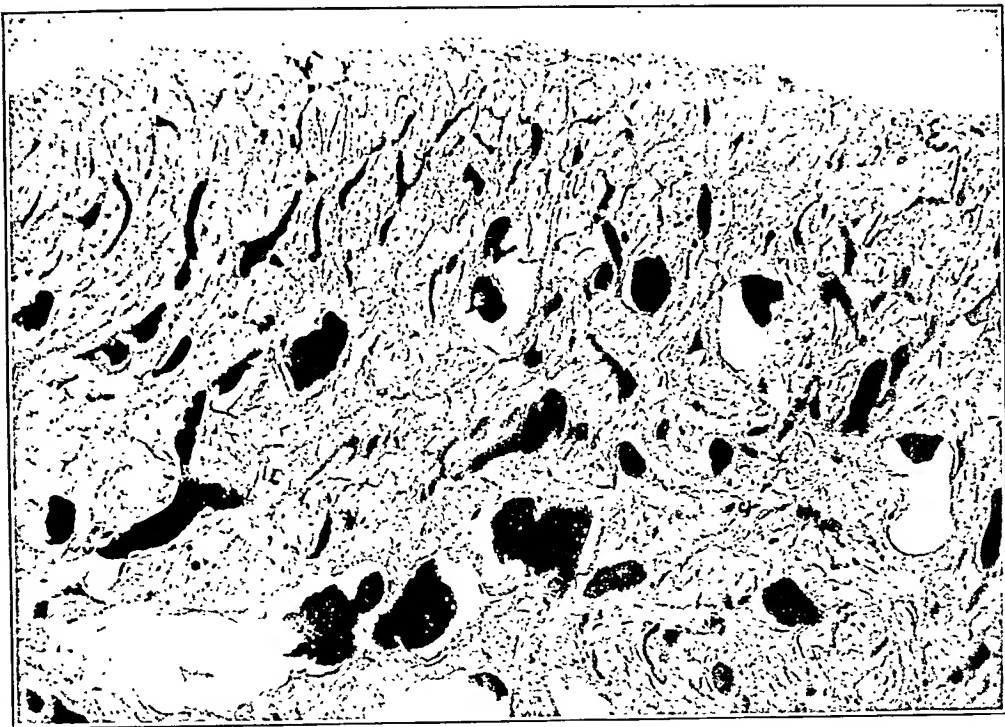


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PLATE 24

FIGS. 14 and 15. Two photomicrographs ($\times 60$) of sections from a series showing the invagination of the endometrial tissue of a direct or primary endometriosis into the lumen of a receiving sinus of the uterine wall (Case 3). The first section shows the relation of the endometrial tubule (E.T.) to a receiving sinus (the latter is not a lymph vessel as it contains a small amount of blood and corresponds in its structure and situation with the receiving sinuses of injected specimens). In the second photomicrograph the same endometrial tubule surrounded by stroma is shown bulging into the lumen of the sinus but covered by the endothelial lining.

FIGS. 16, 17 and 18. Three photomicrographs ($\times 60$) of sections from the same series as those shown in Figs. 14 and 15. The first shows a greater bulging of the tubule (E.T.) into the sinus (compare with Fig. 15). In the second one the tubule lies apparently almost entirely within the lumen of the sinus and without any evidence of the endometrial tissue from which it came. The third photomicrograph shows a cross-section of the tubule surrounded by stroma and covered by endothelium and attached to wall of the sinus by the latter. The tubule surrounded by its stroma is apparently within the lumen of the vessel but is extra- or retro-endothelial and not truly intravascular; its epithelium and stroma are directly continuous with the tubule shown in Figs. 14 and 15. The latter was possibly continuous with a tubule of the mucosa lining the uterine cavity. The endometrial tissue within the sinus is not of metastatic or embolic origin because serial sections showed that both the epithelium and stroma are continuous with similar endometrial tissue outside of the sinus (compare with Figs. 30, 31 and 33 from the same case).



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FIG. 19a. Photomicrograph ($\times 310$) of the parametrium with bits of the uterine mucosa (stroma and epithelium) enmeshed in the tissues of the former. The block of the uterine wall from which the section was made included a portion of the uterine mucosa (menstruating). Bits of the friable uterine mucosa were probably set free in the embedding solutions and some of these became entangled in the loose tissues of the parametrium. Similar pieces of the uterine mucosa, in like manner, might fall into a gaping uterine sinus or vein (see Figs. 19b, 20 and 21).

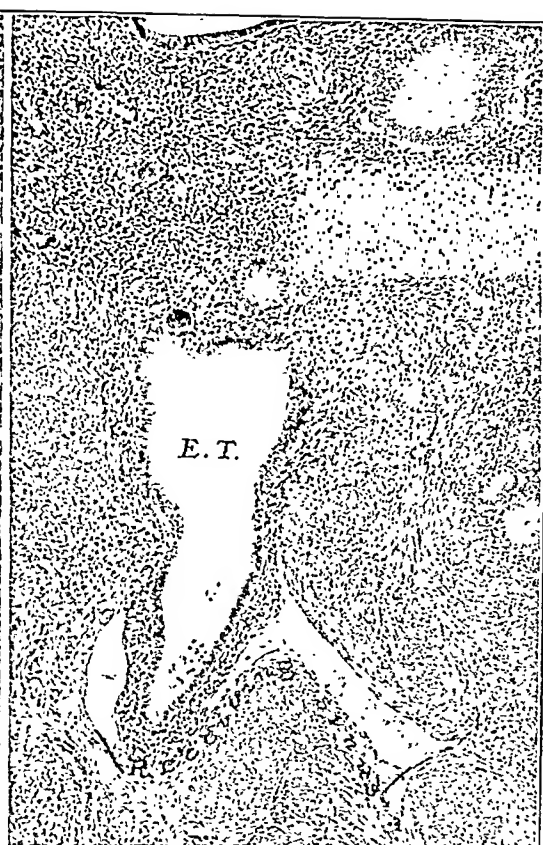
FIG. 19b. Photomicrograph ($\times 310$) of a receiving sinus of a uterine wall showing a strip of "mucosal epithelium" lying free in its lumen; patient menstruating at the time of the operation. The block of the uterus from which the section was made included a portion of the uterine mucosa. Serial sections had been made and the epithelium was not attached to the wall of the sinus. While I cannot exclude its origin from the menstrual dissemination of this bit of the uterine mucosa into the sinus, I believe that it is more apt to be an artefact and that it arose in the same manner as the fragments of mucosa shown in Fig. 19a. Had the epithelium gained access to the sinus before the specimen had been fixed I would expect to find it attached to the wall of the sinus or else with blood about it (see Figs. 25, 27 and 53).

FIG. 20. Photomicrograph ($\times 310$) of a portion of a vein of the uterine wall showing a piece of the uterine mucosa lying free in its lumen; from the same uterus as that shown in Fig. 19a; section taken near the surface of another block. I cannot exclude the origin of this tissue from the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus, but for the reasons given in the legend of Fig. 19b I believe that it also is more apt to be an artefact.

FIG. 21. Photomicrograph ($\times 60$) of a section of an arcuate vein containing both blood and a bit of the uterine mucosa, the latter lying free in the lumen of the vein, from the same uterus as those shown in Figs. 19a and 20 but from a different block of the uterus and from a section taken near the middle of the block. The lumen of the vein is over half filled with blood but the tissue lies free in the empty portion of the vein. It is much more difficult to decide the origin of this bit of tissue. I believe that it also is more apt to be an artefact. A piece of uterine mucosa could drop into the lumen of the unfilled portion of a vein as easily as into an empty one. Had the tissue been embolic from the menstrual reaction it would probably either be partially or wholly embedded in the blood or attached to the lining of the vessels as those shown in Figs. 34, 43 and 53.



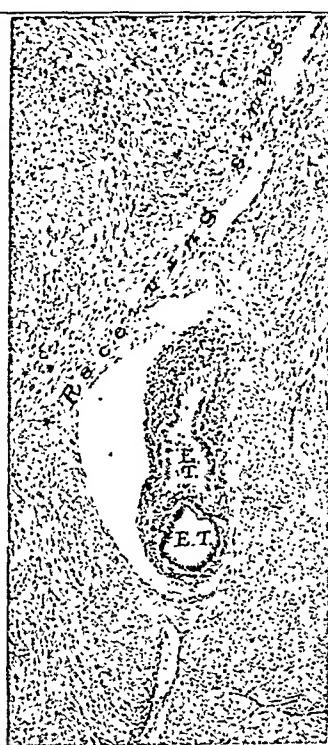
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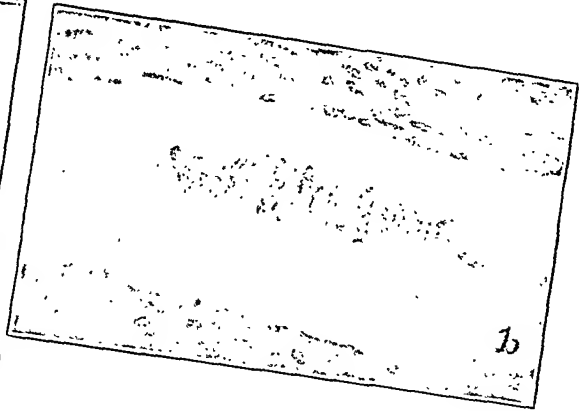
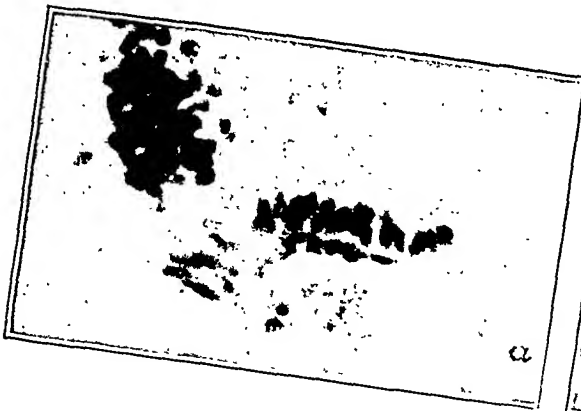


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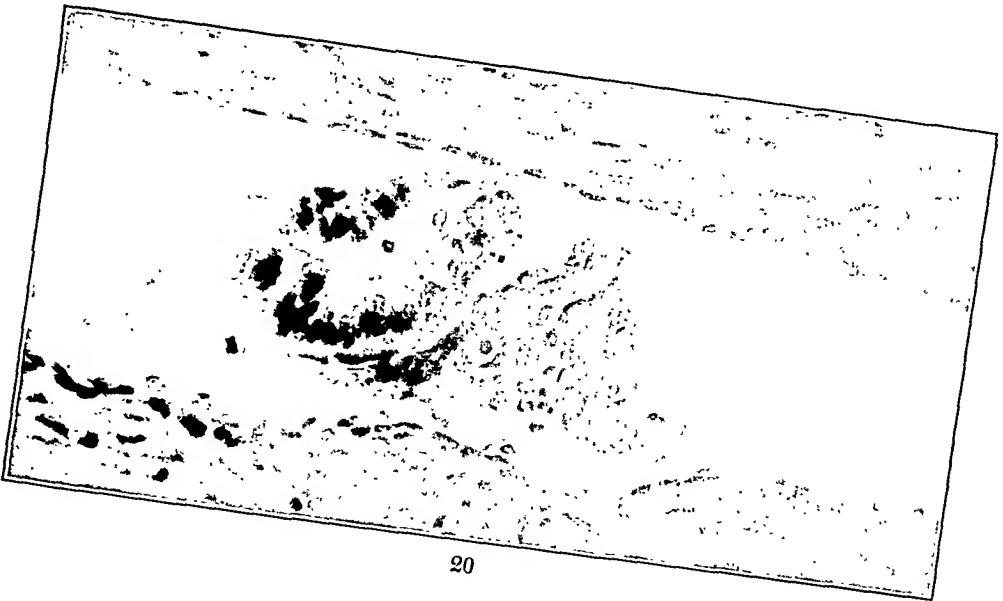
FIG. 22. Photomicrograph ($\times 25$) of the uterine mucosa and underlying muscularis (in the angle between the anterior and posterior uterine wall) showing blood and bits of the uterine mucosa in a large mucosal sinus; second day of the menstrual period (Case 1). The mucosal sinus is unusually large as though two sinuses had become fused. The mucosa over the sinus shows a characteristic reaction of menstruation with the separation of its superficial from its deeper layers by the extravasation of blood into the tissues of the mucosa (Fig. 7) and evidence that blood containing bits of the latter had escaped into the uterine cavity. There is just as strong evidence that some of this extravasated menstrual blood, carrying with it bits of the uterine mucosa, had escaped through the ruptured mucosal sinus into the lumen of the latter (see upper arrow). It is also possible that fragments of the uterine mucosa might be carried from the mucosal sinus into the receiving sinuses of the myometrium and thence into the deeper vessels of the uterine wall (see Figs. 23, 24 and 25). Only the beginning of the receiving sinuses of the myometrium, into which this mucosal sinus empties, appears in this section (see the two lower arrows).

FIG. 23a. Photomicrograph ($\times 25$) showing a mucosal sinus undoubtedly emptying into a receiving sinus. It is a venous sinus and not a lymph vessel because the veins of the uterus had been injected with bismuth.

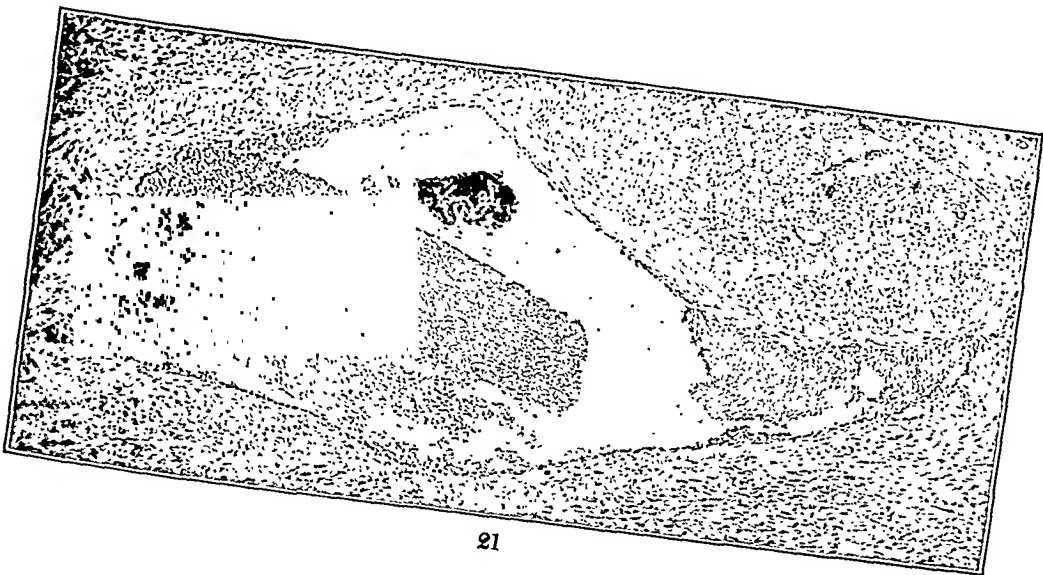
FIG. 23b. Photomicrograph ($\times 25$) of the uterine mucosa and underlying muscularis from a section close to the one shown in Fig. 22. A vessel with the same structure, situation and general course as that shown in Fig. 23a is present and I therefore believe that it also is a venous sinus and not a lymph vessel. This sinus contains a small amount of blood and bits of endometrium (end) in both its mucosal and myometrial portions similar to those shown in the mucosal sinus of Fig. 22. I believe that it is possibly the receiving sinus into which the mucosal sinus, shown in Fig. 22, emptied. It is situated where the latter should be. (Unfortunately, serial sections had not been made. Many sections had been cut from the block and not all of them saved before the condition shown in Fig. 22 had been seen.)



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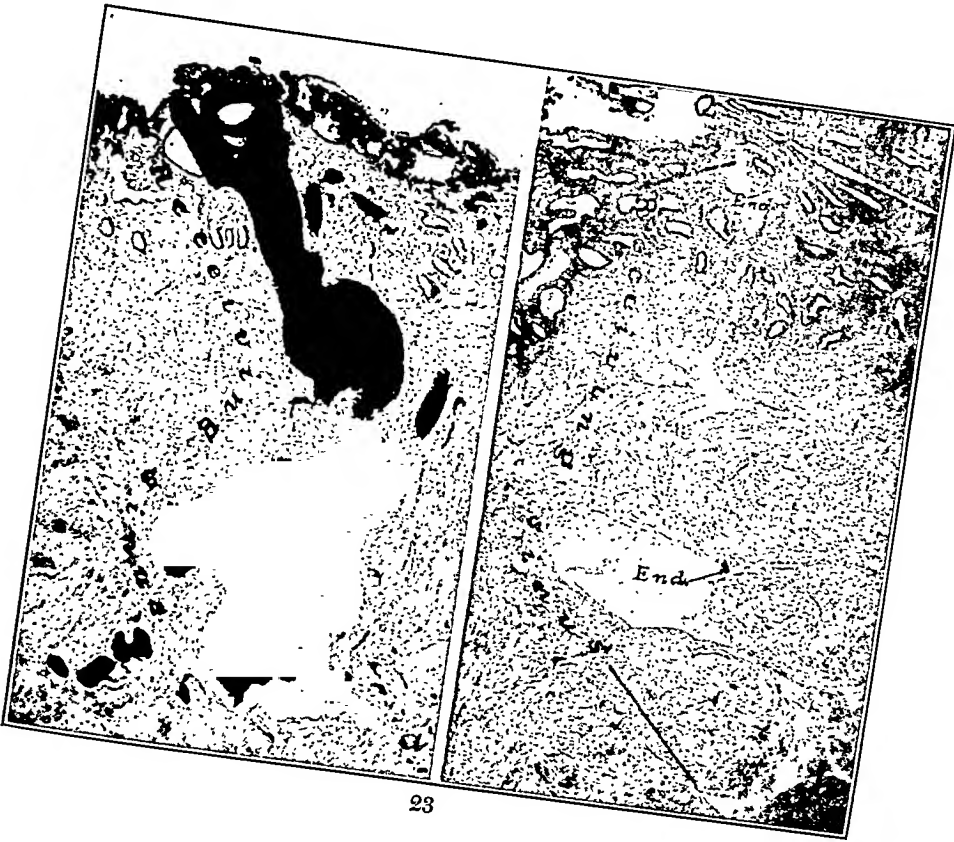
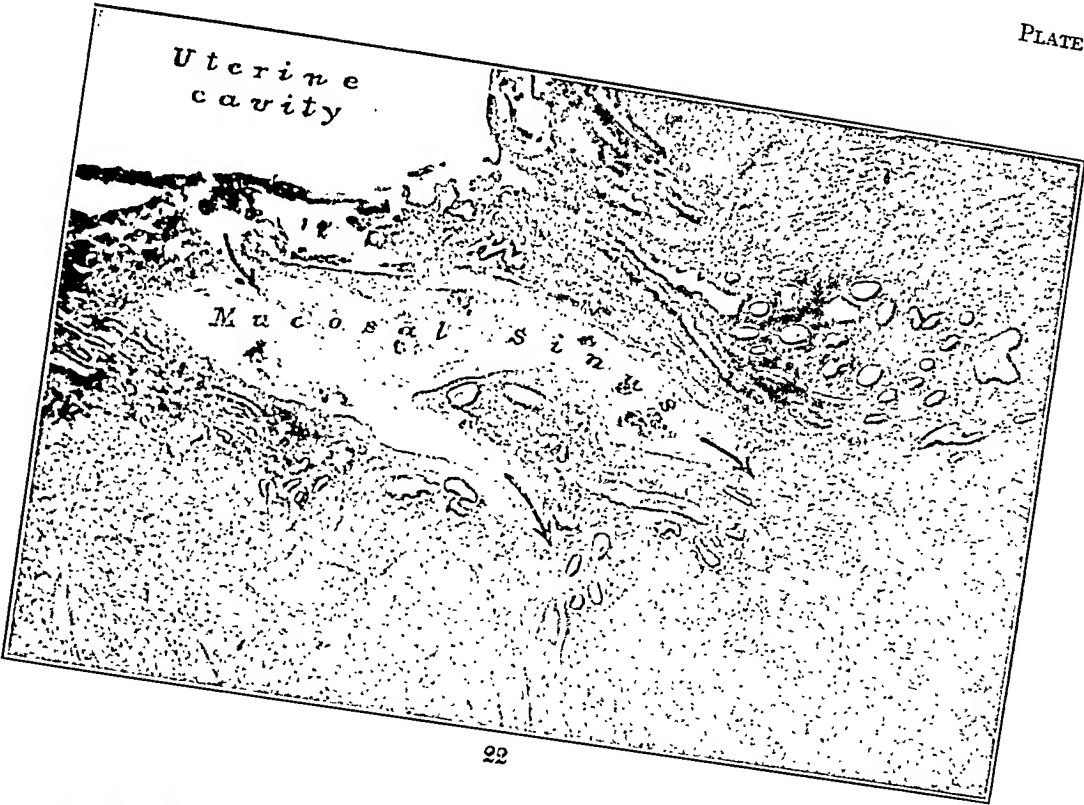
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Metastatic or Embolic Endometriosis

FIG. 24. Photomicrograph ($\times 10$) of a section from the same block of the uterine wall as those shown in Figs. 22 and 23. A receiving sinus is present radiating from the uterine mucosa (the same one shown in Fig. 23*b*). It contains a small amount of blood in places and also small bits of the uterine mucosa. Larger bits of the uterine mucosa are present in the vessels indicated by the pointer "End." (see Figs. 25 and 27). Serial sections were not made and therefore it cannot be proved that this receiving sinus emptied into the vessels containing the larger bits of the uterine mucosa but it probably did.

FIG. 25. Photomicrograph ($\times 60$) of the two vessels containing bits of the uterine mucosa indicated by the pointer "End." of Fig. 24. Other sections demonstrate that these are but two sections of the same vessel. They contain bits of uterine mucosa surrounded by blood and the latter is adherent to the lining of the vessels, thus demonstrating that these fragments gained access to the lumen of the vessel before the tissues were fixed and are not artefacts as those shown in Figs. 20 and 21. The tissue in these vessels has the same histologic structure as that in the extravasated blood of the mucosa lining the uterine cavity, in the mucosal sinus and that in the receiving sinus. I believe that it reached its present situation by menstrual dissemination into the venous circulation of the uterus through the channels indicated in Figs. 22, 23 and 24.

FIG. 26. Photomicrograph ($\times 60$) of a section of the uterine wall showing a mural thrombus attached to the lining of a vessel from the same block of the uterus as the section shown in Fig. 25. This vessel was situated a little deeper in the uterine wall than that shown in Fig. 25. For a higher magnification showing its histologic structure, see Fig. 27*b*.



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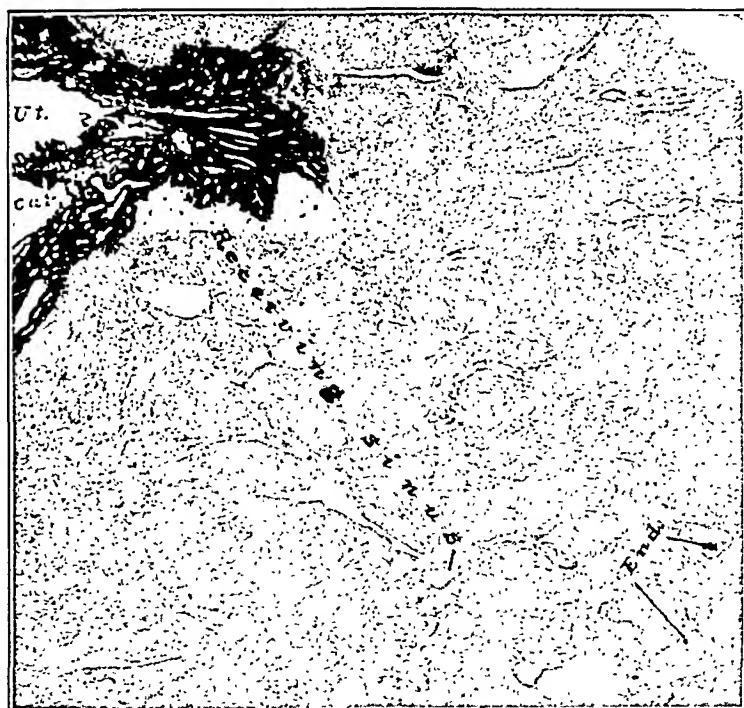
Metastatic or Embolic Endometriosis

FIG. 27a. Photomicrograph ($\times 310$) of the contents of the vessel containing the larger amount of endometrial tissue shown in Fig. 25. It consists of stroma and epithelium in a fair state of preservation and identical in their structure with that of similar bits of endometrial tissue in the extravasated blood of the uterine mucosa and in the mucosal sinus of the section shown in Fig. 22.

FIG. 27b. Photomicrograph ($\times 310$) of the mural thrombus shown in Fig. 26. The thrombus is attached to the endothelial lining of the vessel. It consists of fibrin, leucocytes, epithelium-like cells and two clumps of cells which might be interpreted as degenerating stromal cells. It would seem to represent a later stage of the condition shown in Fig. 27a and might indicate that the "endometrial tissue" in the thrombus is either dead or at least in a poor state of preservation.

FIG. 28. Photomicrograph ($\times 310$) of a portion of a peripheral vein in the fundus of the uterus showing blood and bits of "uterine mucosa" attached to the lining of the vessel (Case 2). The block from which the section was made was cut from the uterus after the latter had been hardened in formalin for a few days. The smaller fragment is adherent to the lining of the vessel while the larger is partially enveloped in blood and fixed to the wall of the vessel, thus demonstrating that they must have reached their present situation before the tissues of the specimen had become fixed. The last menstrual period occurred three weeks before the operation. More sections were made from the same block and a similar condition was found in another vein (see also Fig. 29).

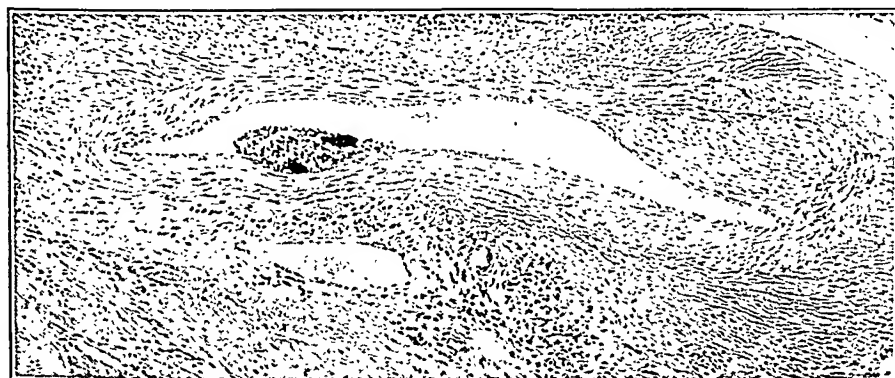
FIG. 29. Photomicrograph ($\times 310$) of a portion of an arcuate vein of the anterior uterine wall from the same uterus as the section shown in Fig. 28 but from a block taken from the uterus at a later date. Over a year after the operation many blocks were taken from the uterus and many sections were studied from each block, and in two vessels of the uterine wall embolic endometrial tissue was found, such as is shown here. The endometrial tissue is adherent to the lining of the vessel and the former stains poorly compared with the mucosa lining the uterine cavity (in the same section) thus suggesting that the former had undergone degenerative changes. The findings in these two cases demonstrate that bits of the uterine mucosa escape into the venous circulation of the uterus during menstruation and suggest that they may be retained in the sinuses of the uterine wall. They also suggest that this tissue may not always live.



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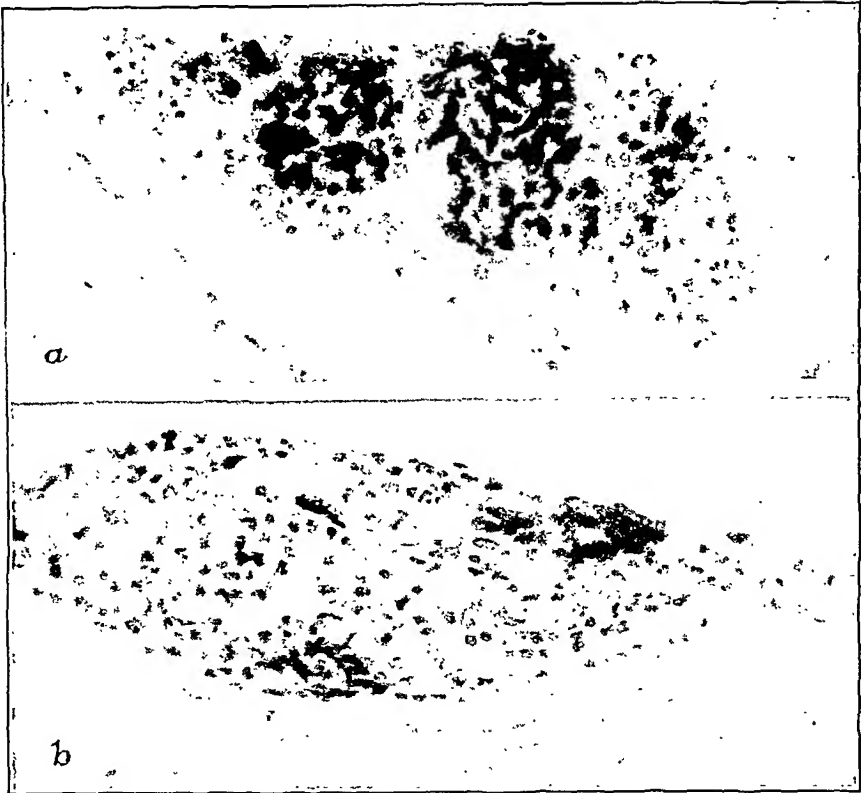


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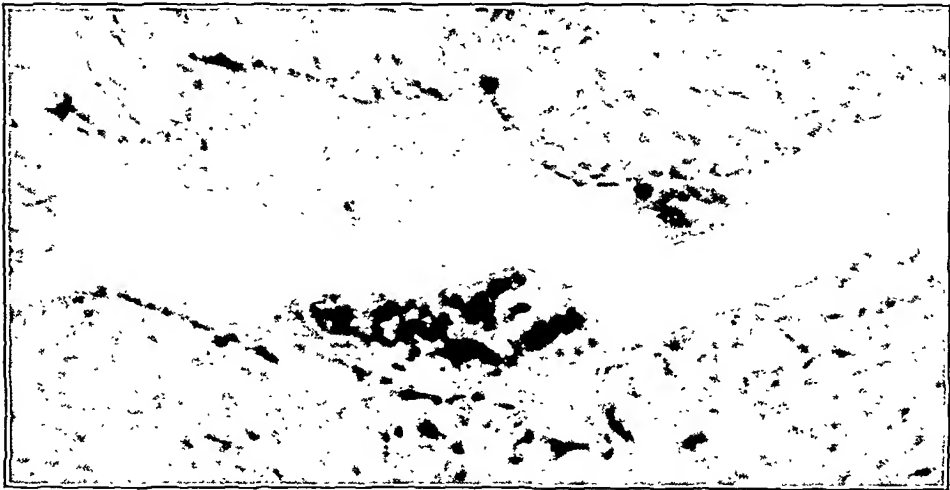
FIG. 30. Photomicrograph ($\times 20$) of a section of the uterine wall, including its mucosa, demonstrating an embolus-like piece of the latter (M) in a receiving sinus (Case 3). Serial sections had been made of this portion of the block and it was shown that this receiving sinus extended into the mucosa and that the "embolus had lodged" at the site of a branching of the sinus. An endometriosis (End.) is present in this section of the uterine wall which, from an extra-endothelial position, bulged into a branch of the receiving sinus, as shown by sections of the block taken at another level and similar to the lesion shown in Fig. 14.

FIG. 31. Five photomicrographs ($\times 60$) of sections showing the appearance of the endometrial tissue in the receiving sinus of Fig. 30, at different levels of the series. *a* shows a bit of stroma apparently adherent to the lining of the sinus but really not attached. *b* (from the same section shown in Fig. 30) demonstrates epithelium arranged in the form of a gland and surrounded by stroma. This tissue apparently lies free in the lumen of the sinus at this level as does also the tissue shown in *c*. *d* demonstrates that the bit is really a polyp, attached by a slender pedicle to the wall of the sinus (for a higher magnification of this polyp see Fig. 33). *e* shows the last appearance of the epithelium in the polyp with a fragment of stroma to the left of it. The series of sections from which these were chosen demonstrated that an endometrial polyp was present in this sinus attached by a pedicle to the wall of the latter and apparently surrounded by endothelium just as similar polyps arise from the invasion of endometrial tissue into a vessel pushing the endothelium ahead of it (see Figs. 14 to 18 inclusive, from the same case) but differing from the latter in that it was conclusively shown that the gland lay entirely within the sinus and was not a cross-section of a tubule continuous with a similar structure outside of the vessel.

FIG. 32. Four photomicrographs ($\times 60$) of sections showing embolic "uterine" epithelium attached by fibrin to the wall of a receiving sinus. From the same block of the uterine wall as the sections shown in the preceding illustrations but at a different level and in another portion of the block. Serial sections demonstrated that the epithelium, arranged in the form of a gland, lay entirely within the sinus, was not covered by endothelium and was not continuous with any endometrial tissue outside of the sinus. It is attached to the wall of the sinus by fibrin, thus demonstrating that it reached its present situation before the tissues had been fixed, either from the trauma of the operation, the incision of the uterus immediately after the operation or the menstrual dissemination of this tissue into the sinus (patient flowing at the time of the operation). For a higher magnification of the lesion in *c* see Fig. 34.



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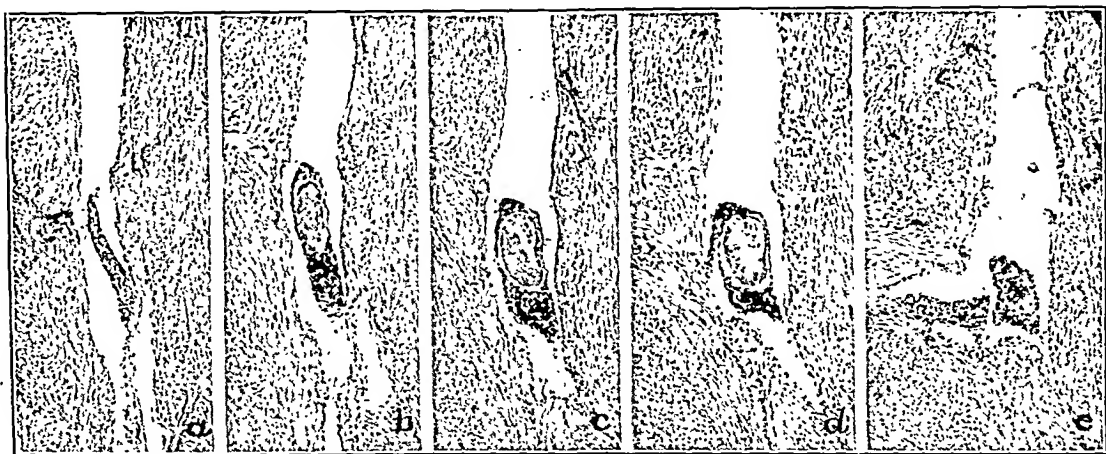
FIG. 33. Photomicrograph ($\times 310$) of the endometrial polyp shown in *d* of Fig. 31. It consists of an epithelial gland surrounded by endometrial stroma and the latter apparently covered by endothelium; it is extra- or retro-endothelial and is attached to the wall of the sinus by a slender pedicle. This endometrial polyp must have arisen either from a metaplasia of the endothelial lining of the sinus, an implantation of a bit of the uterine mucosa escaping into the sinus during menstruation (Fig. 31), an implantation from the menstrual reaction of the heterotopic endometrial tissue bulging into a branch of the sinus (see Fig. 31) but separated from the lumen of the sinus by its endothelial lining, or if it previously had been continuous with the latter this connection in some way must have been severed. For a possible explanation of its origin see Fig. 34.

FIG. 34. Photomicrograph ($\times 310$) of the "uterine" epithelium attached to the wall of the sinus by fibrin (Fig. 32*c*). The epithelium is arranged in the form of a gland and is entirely intravascular. Its possible origin was discussed in the legend of Fig. 32. If it is a menstrual embolus, as well it might be, the origin of the polyp shown in Fig. 33 can readily be explained. Should the endothelium of the sinus cover such an implant and the endometrial tissue live, it might readily develop into a polyp similar to that shown in Fig. 33 or a lesion similar to that shown in Fig. 35.

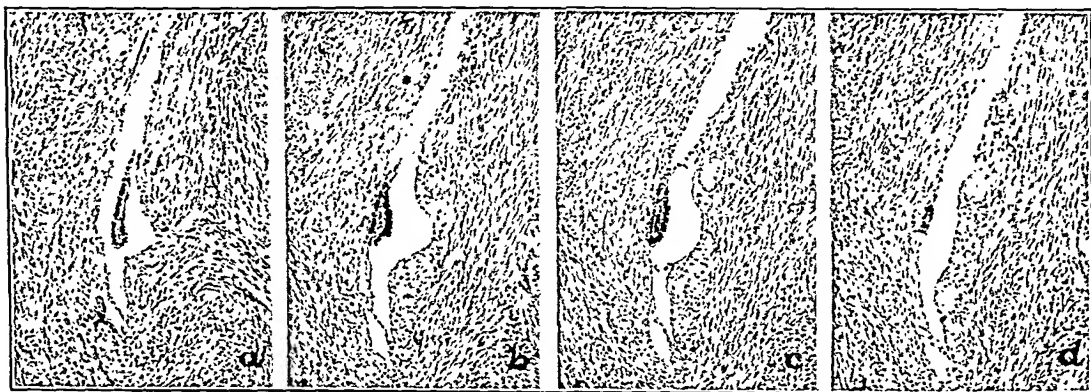
FIG. 35. Photomicrograph ($\times 310$) of a portion of a sinus, a branch of the sinus shown in Figs. 30 and 31, with extra-endothelial endometrial tissue bulging into its lumen. Serial sections demonstrated that this endometrial tubule was not continuous with the gland in the polyp shown in Fig. 33. The series beyond this portion of the block was incomplete and the relation of this endometrial tissue to that outside of the sinus was not determined. I believe that it might have been continuous with the latter. If true, the latter might have arisen from this and not the reverse. The conditions shown in Figs. 33 and 34 suggest that this endometrial tissue might have developed from the implantation of embolic endometrial tissue with a subsequent growth of the endothelium of the sinus over it.



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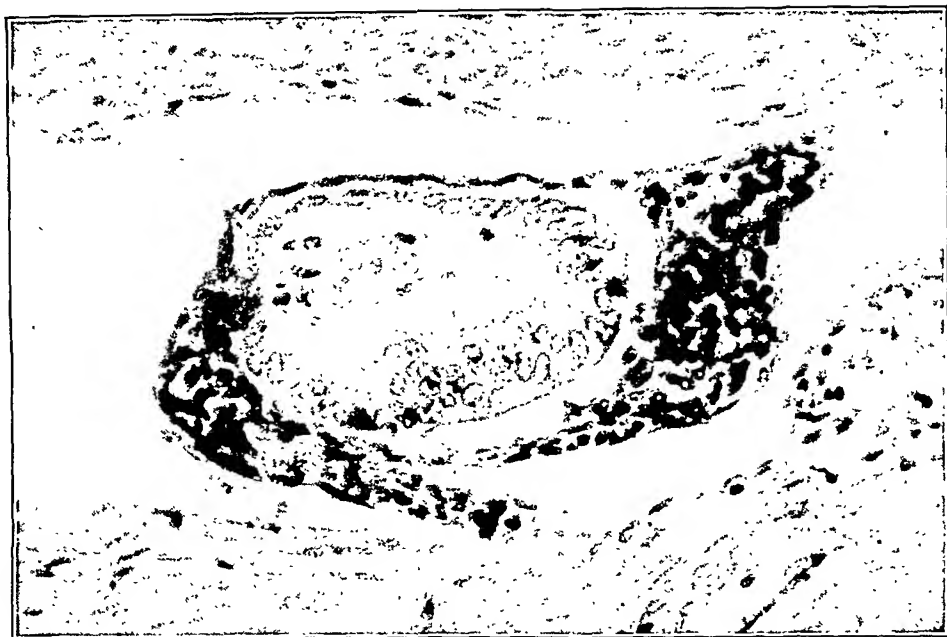
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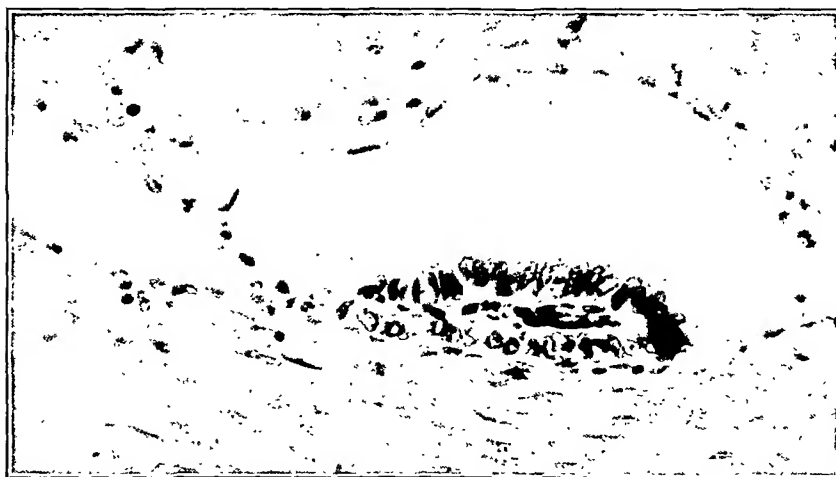
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FIG. 36. Photomicrograph ($\times 5$) of a section of the left half of the posterior uterine wall (Case 4). An endometriosis is present, most marked in the radial zone. The distribution of the endometrial tissue in that zone is similar to the distribution of the bismuth in corresponding sections of uteri in which the veins have been injected. In this section many of the spaces occupied by the veins and venous sinuses of the uterine wall are filled with endometrial tissue (Fig. 38). An apparent receiving sinus (Fig. 39) is well outlined by this tissue. The entire uterus was cut into blocks and many sections were studied from each block and in only a small area was an invasion of the uterine wall by its mucosa found. I believe that the endometriosis of the radial zone shown in this photomicrograph possibly arose from this invasion. The endometriosis of the peripheral zone was more likely of embolic or metastatic origin. While the patient was menstruating at the time of the operation, only a very few of the areas of ectopic endometrial tissue showed a reaction to menstruation (Fig. 40).

FIG. 37. Photomicrograph ($\times 5$) of a section of the right half of the posterior uterine wall at about the same level as that shown in Fig. 36. An endometriosis is not present in the radial zone of this section. An embolic implantation of endometrial tissue (End.) is situated in the arcuate veins (Figs. 49 and 50) and small emboli of endometrial tissue (E,E) are present in the veins of the peripheral zone (Fig. 51). There were two very interesting features of this uterus; one was the presence of an endometriosis of the direct type in the left half of the posterior uterine wall with only a very few evident embolic lesions and those in the peripheral zone; the other was multiple embolic lesions in the right half of the posterior uterine wall, most numerous in the peripheral zone with only a slight invasion of that half of the posterior uterine wall by the endometrial tissue from the opposite side.



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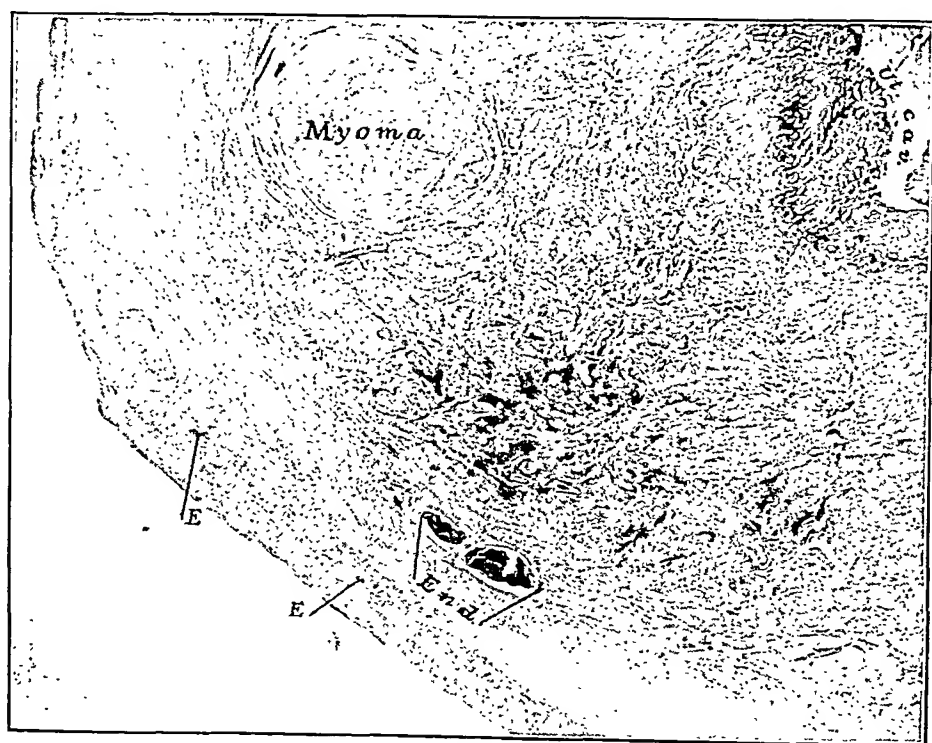
FIG. 38. Photomicrograph ($\times 60$) of a portion of the section shown in Fig. 36 illustrating a frequent type of lesion found in a direct or primary endometriosis. No trace of a vessel or sinus is evident in or about the endometrial area to the left. I believe that it fills the space originally occupied by a sinus and has either obliterated the lumen of the latter or pushed it to one side. The endometrial area to the right, a continuation of the former, projects into the lumen of a sinus like a sessile polyp but the surface of the latter is covered by endothelium. The endometrial tissue is invading the space occupied by the sinus in a retro-endothelial course, as has been so well described by Robert Meyer. I believe that this vessel is probably a venous sinus and not a lymphatic. It is conceivable that, should this endometrial tissue react to menstruation, blood containing bits of endometrial tissue would escape into the lumina of its tubules (see Fig. 40) and at times might rupture the overlying endothelium and escape into the lumen of the sinus.

FIG. 39. Photomicrograph ($\times 25$) of a portion of the "receiving sinus" shown in Fig. 36. The space occupied by the sinus is partially filled by endometrial tissue. The endometrial tissue has apparently invaded this sinus in a retro-endothelial course distorting the lumen (L.) of the latter as shown in Fig. 38 and in the manner of a direct or primary endometriosis. A direct invasion of this portion of the uterine wall by its mucosa was found and this is possibly a continuation of it. On the other hand, its histologic structure suggests a "canalized" endometrial thrombus which might have arisen from the implantation and growth of an embolus of endometrial tissue on the lining of the sinus with subsequent covering by endothelium. As will be shown, it is possible for the endothelium of a vessel or sinus to grow over endometrial tissue implanted in that vessel and give rise to lesions somewhat similar to those shown in this and the preceding illustration (see Figs. 41, 42 and 50).

FIG. 40. Photomicrograph ($\times 60$) of a section of a menstruating area in the endometriosis shown in Fig. 36. An endometrial cavity (dilated tubule) is filled with blood and bits of endometrial tissue. This blood might extend through the lumen of the tubule to other parts of the uterine wall invaded by this tissue. The endothelial lining of a venous sinus in this area or adjacent to it might be ruptured by the menstrual reaction, and menstrual blood carrying with it bits of the uterine mucosa, might escape into the venous circulation of the uterus. I have not been able definitely to demonstrate this in the menstrual reaction of endometrial tissue of a direct endometriosis but am confident that it does take place. For anatomic and physiologic reasons it should occur and I have observed it in an endometriosis of the posterior vaginal wall (Figs. 60 and 61).



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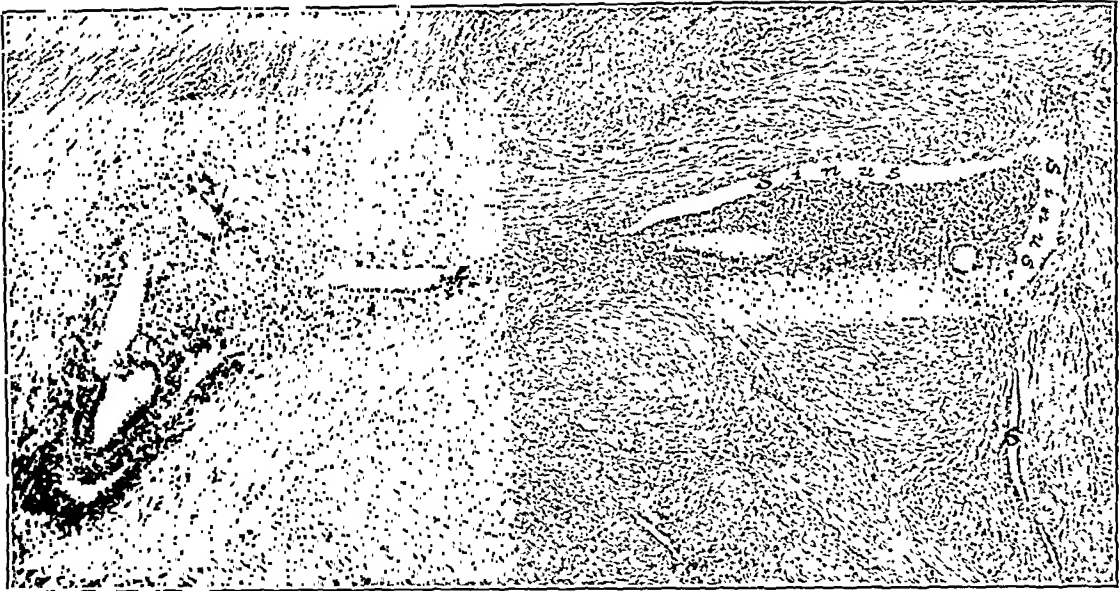


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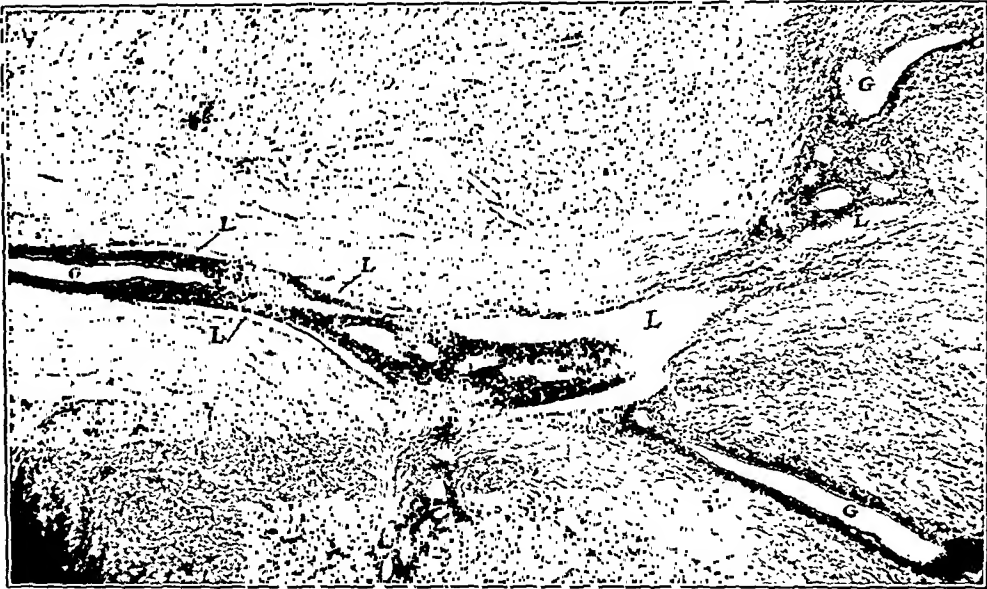
FIG. 41. Photomicrograph ($\times 130$) of an oblique section of a sinus almost completely filled with endometrial tissue (from the peripheral zone of the left half of the posterior uterine wall near the midline). Sufficiently complete serial sections were made to demonstrate that this tissue was not continuous with that in the radial zone or any outside of the sinus, but arose either from an implantation of a bit of endometrial tissue on the lining of this sinus or else from a metaplasia of its endothelial lining. I believe the former (see Figs. 42 and 43). In this section the implant is not attached to the wall of the sinus at A and B. The endometrial cavity, lined by epithelium, communicates with the lumen of the sinus at A. Should menstrual blood escape into this endometrial cavity, as shown in Fig. 40, it might carry with it bits of endometrial tissue into the lumen of the sinus and thence into the venous circulation of the uterus. At B the implant bulges into the lumen of the sinus but its surface is covered by endothelium, growing over it from that lining the sinus. This implantation lesion differs from that arising from the invasion of a sinus by endometrial tissue from without in that it is primarily intravascular while the latter is extravascular (retro-endothelial). Should complete endothelialization of an implant occur, it might be histologically indistinguishable from that of a direct invasion and in its subsequent growth it might invade the lumen of the sinus as a retro-endothelial course and thus give rise to lesions similar to those shown in Figs. 36, 38 and 39. I have not been able to prove that this occurs.

FIG. 42. Photomicrograph ($\times 130$) of the same sinus shown in Fig. 41 but at one end of the implant. At the right, the endometrial tissue is grafted on the wall of the sinus. To the left it is attached to the wall of the sinus by fibrin and endothelium apparently has begun to cover it. While the lesion shown here probably is an extension of the endometrial tissue shown in Fig. 41 it could well represent a stage in the implantation of embolic endometrial tissue lodging in a sinus (see Fig. 43).

FIG. 43. Photomicrograph ($\times 130$) of the same sinus shown in Figs. 41 and 42 but beyond the implant. The sinus is partially filled with blood containing free bits of endometrial tissue possibly cast off by menstruation from the implant or from endometrial tissue elsewhere in the uterus as indicated below. Should this tissue become attached to the wall of this or another sinus and live, the lesions shown in Figs. 41 and 42 might arise. I believe that the lesion shown in Fig. 41 arose in this manner — from embolic endometrial tissue either cast off from another implant, from the menstrual reaction of a direct endometriosis with rupture into a vein, or from the menstruating uterine mucosa disseminating bits of endometrial tissue into the venous circulation of the uterus.



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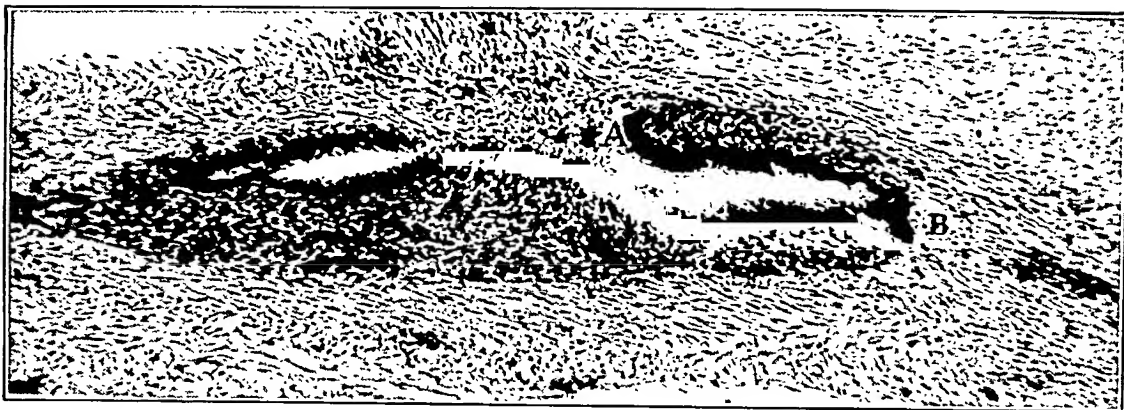
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FIG. 44. Photomicrograph ($\times 25$) of an arcuate vein in the lower portion of of the posterior uterine wall. The lumen of the vessel is almost completely filled with endometrial tissue. It resembles the lesion of a direct endometriosis but is also similar to that shown in Fig. 41. This was the first section obtained after trimming the block and therefore it was impossible to ascertain whether or not it is of embolic origin. My reaction is that it is of embolic origin. This lesion was followed through the block until it disappeared (see Figs. 45 and 46).

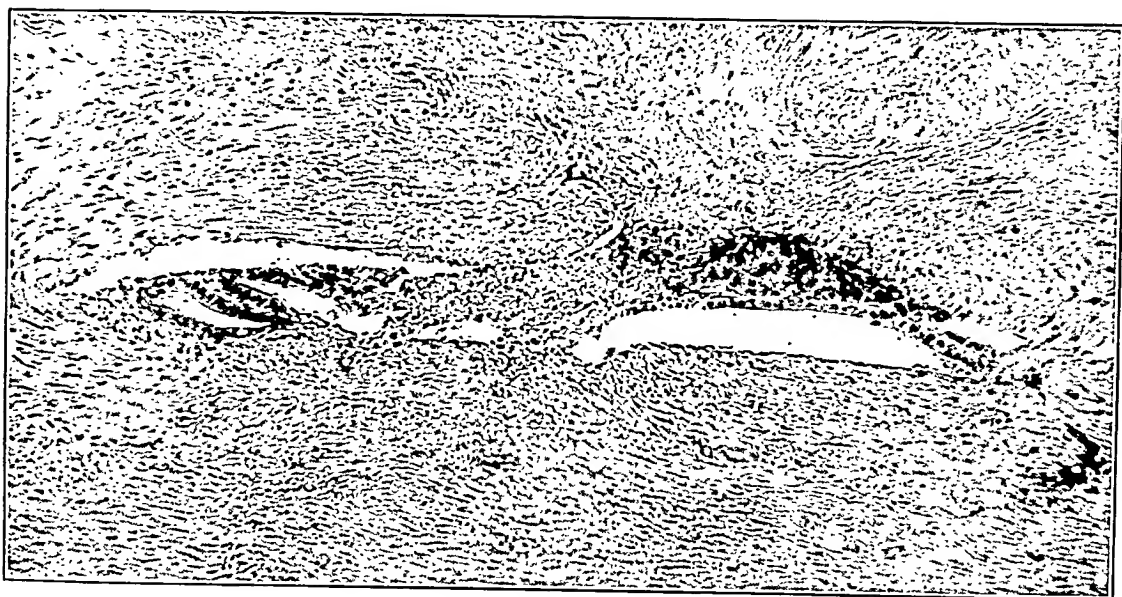
FIG. 45. Photomicrograph ($\times 25$) of the same vein shown in Fig. 44 but deeper in the block. It differs from the latter in that the lumen of the endometrial tubule (cavity) communicates with that of the vein at A and also at B. The latter might be an artefact (torn in cutting) but not the former (see Fig. 47).

FIG. 46. Photomicrograph ($\times 25$) of the vein or branches of the same shown in Fig. 44. A little beyond this level the lesion disappeared. The lesions are similar to those shown in Fig. 38 and circumstantial evidence indicates that the latter might have arisen from the direct invasion of the uterine wall by its mucosa.

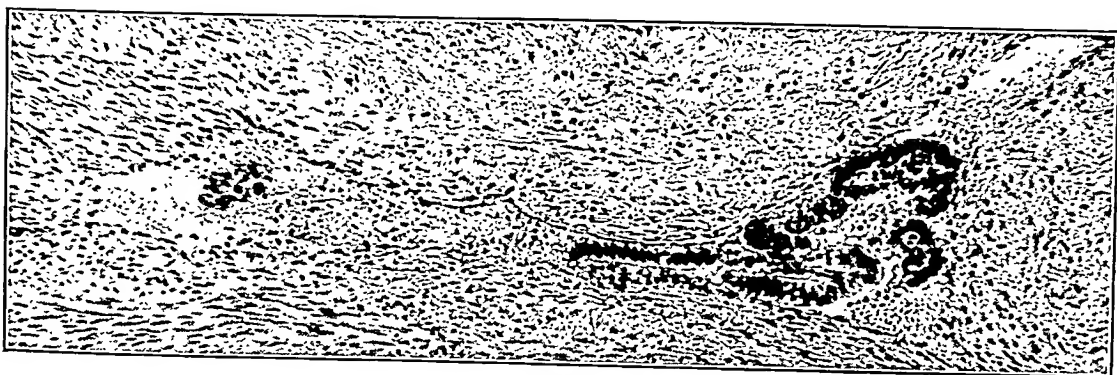
FIG. 47. Photomicrograph ($\times 130$) of the portion of the vein indicated by A of Fig. 45. The endothelial lining of the vessel is well shown in the upper portion of the right half of the photomicrograph. The lumen contains blood and is loosely filled with stroma. The endometrial cavity to the left empties into the lumen of the vein. It is not an artefact. It represents either the incomplete grafting of an endometrial implant as shown in Fig. 41 or else the endothelial covering of the endometrial tissue of a direct endometriosis has been destroyed by menstruation. My present reaction is that it is the former (compare with Fig. 41 which we know is of embolic origin).



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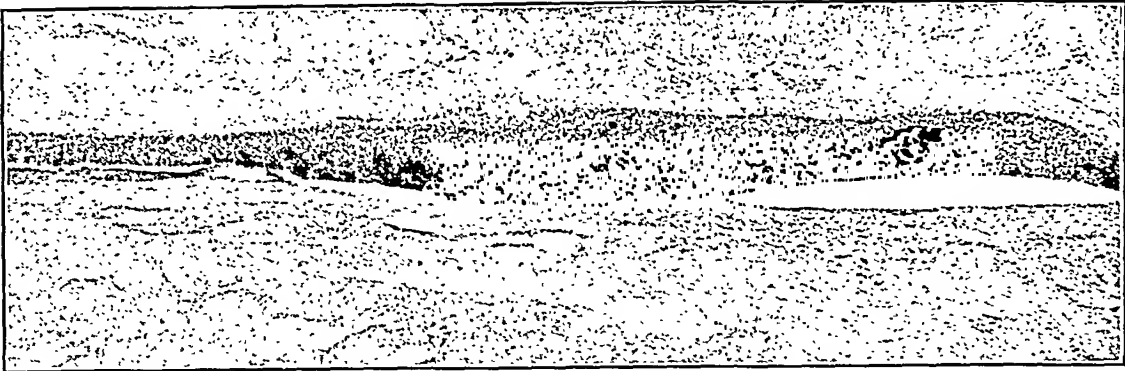
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FIG. 48. Photomicrograph ($\times 25$) of a section of the uterine wall showing an arcuate vein cut obliquely; veins injected with bismuth. The blood from the peripheral and radial zones of the uterus empties into the arcuate veins and is conveyed by these to the venous circulation outside of the uterus. Bits of endometrial tissue carried by menstrual blood into the ruptured venous sinuses of the uterine mucosa (Figs. 22 and 23) would reach these veins through the receiving sinuses (Figs. 9 and 10). These bits might become implanted in these veins (Fig. 49) or under the varying changes in the pressure of the venous circulation of the uterus they might be carried into vessels of the peripheral zone (Figs. 11, 51 and 65) or even escape into the venous circulation outside of the uterus.

FIG. 49. Photomicrograph ($\times 25$) of a section of the arcuate vein shown in "End." of Fig. 37. It is almost an exact duplication of the vein shown in Fig. 48 except that the injection mass is replaced by endometrial tissue. Sufficiently complete serial sections were made of this block to demonstrate that the endometrial tissue in the vein did not arise from the invasion of the vessel by endometrial tissue outside of it. The endometrial tissue in this section is entirely intravascular and must have arisen either from a "metaplasia" of the endothelium of the vessel or else through the implantation and growth of an endometrial embolus similar to those found floating about in lumina of other vessels (see Figs. 43, 53 and 65). The endometrial tissue of this implant has the same structure as that of the mucosa lining the uterine cavity (Fig. 55) and that found in the endometriosis of the opposite side of the posterior uterine wall shown in Figs. 36, 38 and 39.

FIG. 50. Photomicrograph ($\times 25$) of another section of the arcuate vein shown in Fig. 49. The lesion differs from the preceding one in a very important feature. The endometrial tissue in the former is entirely intravascular while that in this section is partially retro-endothelial due to the growth of the endothelium of the vessel over the end of the endometrial tissue projecting into the lumen of the vein (to the left) just as endothelium covers a mural thrombus. Should the endothelium grow more rapidly than the endometrial tissue, it might cover the entire unattached portion of the endometrial implant. Should bits of endometrial tissue be cast off by the menstrual reaction of such an implant they would escape into the venous circulation of the uterus. Some of the endometrial emboli in this specimen might have had such an origin (Fig. 65).

FIG. 51. Photomicrograph ($\times 25$) of a section of the peripheral zone of the uterine wall from the same block shown in Fig. 37 and very near the latter. Bits of endometrial tissue (End.) are present in the veins of this section, some lying free in the lumen of the vessel and others attached to its wall. (For a higher magnification of similar lesions in another section of the peripheral zone see Fig. 65.) These bits of endometrial tissue must have arisen from the dissemination of similar tissue into veins of the peripheral zone from the menstrual reaction of an endometrial implant such as that shown in Figs. 41 and 49, or from the menstrual reaction of the uterine mucosa discharging menstrual blood into a ruptured mucosal sinus as has been demonstrated, or from a similar reaction of the endometrial tissue of a direct endometriosis which must occur but which I have not been able definitely to prove.



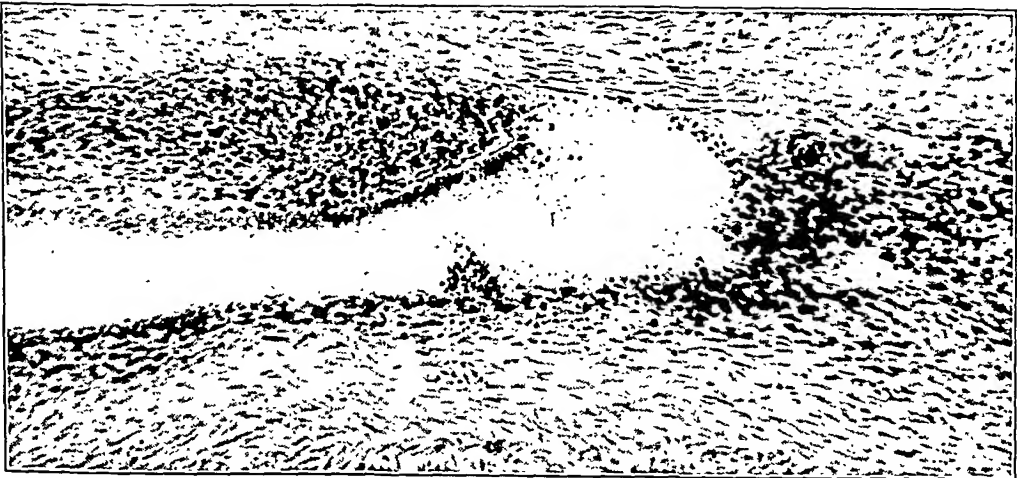
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FIG. 52. Four photomicrographs ($\times 60$) from a series of sections showing the appearance, at different levels, of an embolic implantation of endometrial tissue in a vein or sinus of the peripheral zone of the right half of the posterior uterine wall. *a* shows the condition found near one end of the "endometrial plug" and *d* that found near the other end. In *a* and *d* there is no evidence of endothelialization while in *b* and *c* the implant is apparently partially covered by endothelium. Many lesions similar to this one were found in the peripheral zone of the right half of the posterior uterine wall.

FIG. 53. Three photomicrographs of sections of veins in the right half of the posterior uterine wall. *a* ($\times 60$) shows a bit of endometrial tissue surrounded by blood in the lumen of a large vein near the lateral surface of the uterus, very close to the uterine plexus and therefore almost in the venous circulation outside of the uterus by which it could easily be carried to the lungs. *b* ($\times 130$) shows a bit of uterine epithelium attached to or resting on the lining of a sinus of the radial zone. It is situated between the mucosa lining the uterine cavity and the arcuate veins and is in a channel by which endometrial tissue escaping from the uterine mucosa would reach these veins. *c* ($\times 60$) shows an embolic endometrial implant in a sinus of the peripheral zone with no evidence of endothelialization.

FIG. 54. Photomicrograph ($\times 25$) of an embolic growth of endometrial tissue in a receiving sinus of the right half of the posterior uterine wall. It more than suggests that this endometrial tissue primarily escaped into this sinus from a sinus of the uterine mucosa and that similar bits might have reached the arcuate veins into which the receiving sinuses empty and from the arcuate veins escaped into the peripheral veins of the uterine wall. I believe that the embolic lesions of endometrial tissue in the veins and sinuses of the right half of the posterior uterine wall primarily arose from the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus rather than from a similar reaction of the endometrial tissue of a possible direct endometriosis.



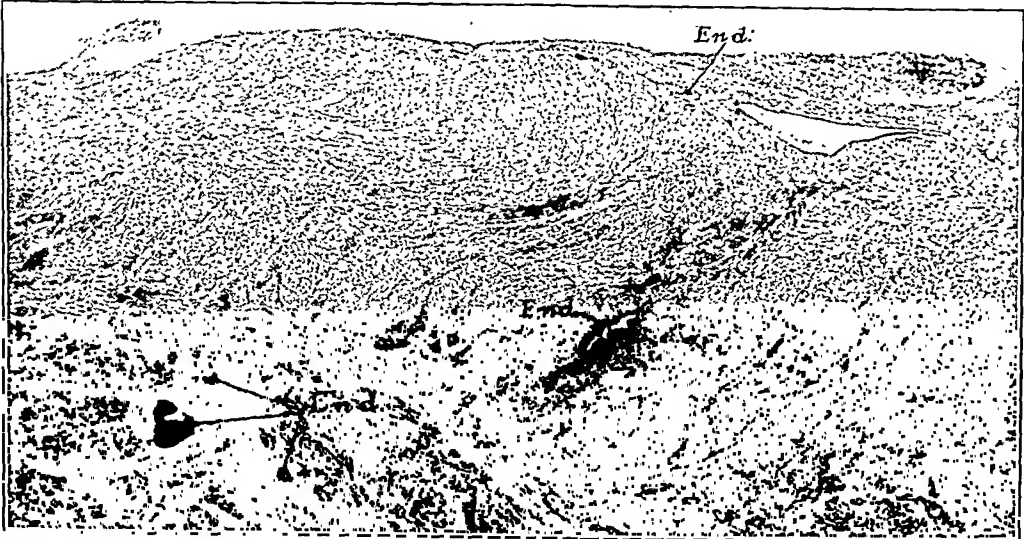
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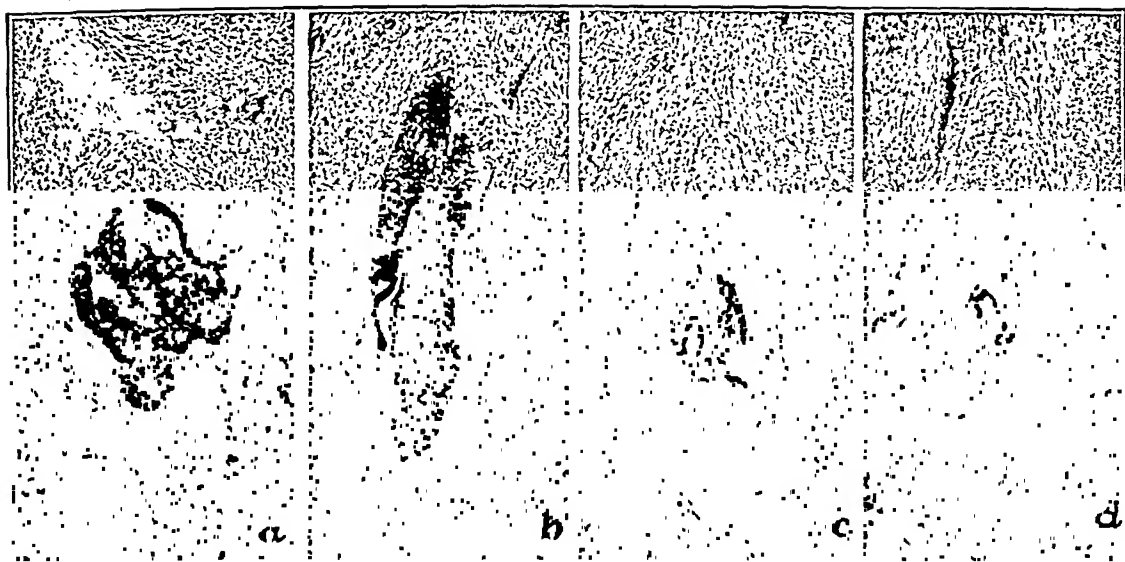
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FIG. 55. Photomicrograph ($\times 25$) of the menstruating mucosa lining the uterine cavity (Case 4). A mucosal sinus is present containing blood and a bit of endometrial tissue (End.). It might be argued that the latter is an artefact in this instance and gained access to the lumen of the sinus from the trauma of the operation or in cutting the blocks from the hardened specimen. Should such a bit escape into the lumen of a mucosal sinus during menstruation, as they do, we might expect to find similar fragments in the sinuses of the uterine wall including the receiving sinuses and also in the arcuate veins and even those of the peripheral zone. As has been shown, not only were these found but also the actual implantation and growth of this tissue in these vessels, thus demonstrating that bits of endometrial tissue disseminated by menstruation are sometimes alive and capable of becoming implanted on the endothelial lining of the veins and venous sinuses of the uterine wall.

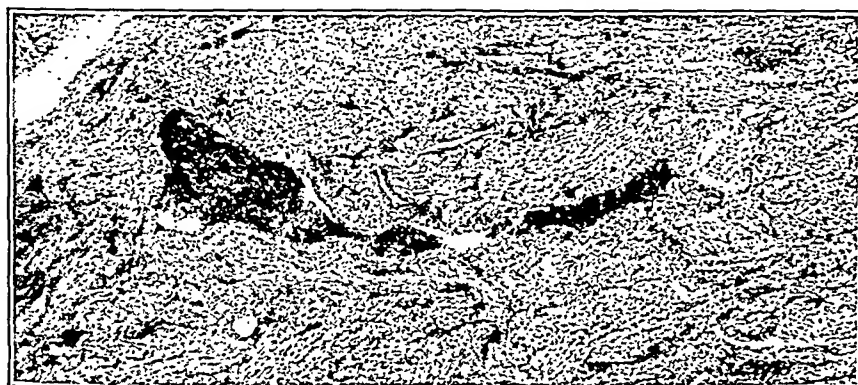
FIG. 56. Photomicrograph ($\times 10$) of a section of a portion of the anterior uterine wall near one of the cornua with the anterior layer of the broad ligament fused to it by the endometriosis in this situation. The anterior cul-de-sac was partially obliterated by a peritoneal endometriosis fusing the anterior surface of the uterus with the anterior layer of the broad ligament and the peritoneum covering the bladder. The lesion was most marked about the uterine cornua. The lumen of the cul-de-sac is patent at both ends of the section ($a - a$ and $b - b$) but is occluded in the center. It would seem that the lesion started at X possibly on or near the peritoneal surface of the uterus and radiated in all directions invading the uterine wall and the anterior layer of the broad ligament to about the same depth. The entire uterus was cut into blocks and many sections were studied from each block. No endometrial tissue was found in the anterior uterine wall except similar to that indicated in this section. Definite embolic lesions, like those in the right half of the posterior uterine wall, were not found in any portion of the lesion. The endometrial lesions in this section and in all others from this portion of the specimen showed an endometriosis of the invasive type apparently spreading from or near the peritoneal surface of the uterus. In a few areas a possible communication of the lumen of an endometrial tubule with that of a vessel was suggested, thus indicating a possible metastatic origin. Three theories may be considered for the origin of the endometrial tissue in this situation. 1. From the stimulation of potential endometrial tissue in the serosa. 2. From the implantation of endometrial tissue on the peritoneum from menstrual blood escaping into the cul-de-sac. Both tubes were patent and endometrial tissue was not found in the ovaries. 3. From endometrial emboli lodging in subperitoneal vessels of the peripheral zone of the anterior uterine wall. I have been unable to determine its origin but believe that primarily it must have been either an implantation or an embolus.



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PLATE 38

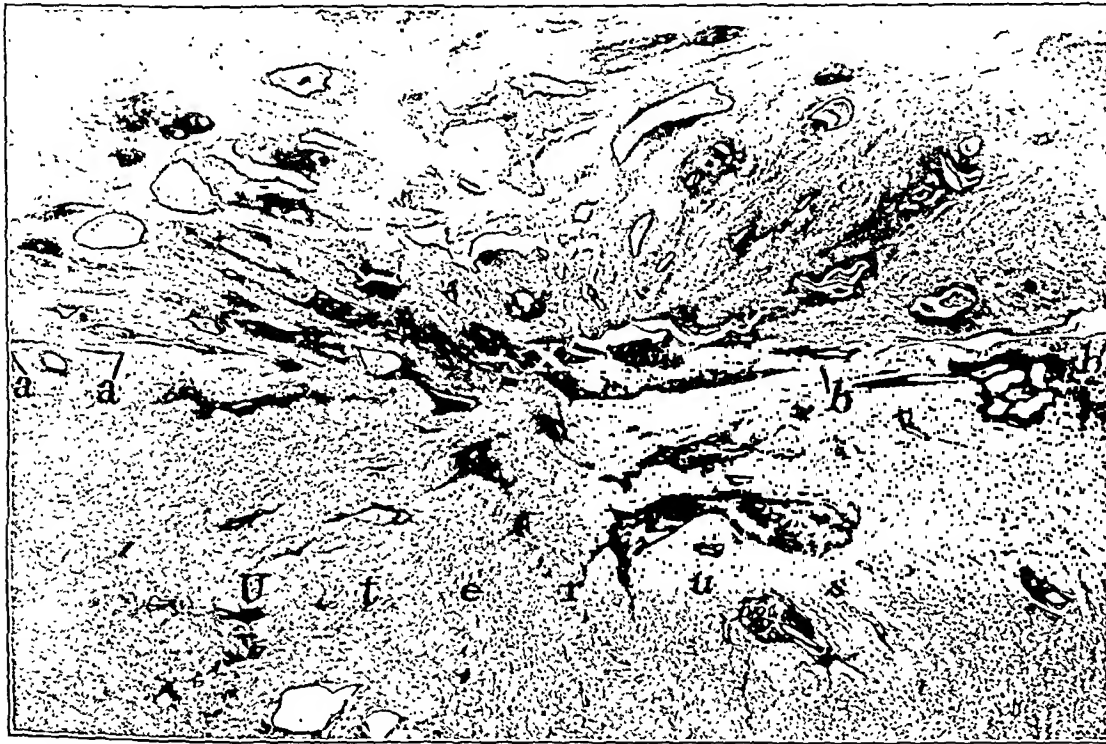
FIG. 57. Photomicrograph ($\times 10$) of a longitudinal section of a portion of the posterior vaginal wall near the cervix (Case 4). An endometriosis is present showing a variety of endometrial lesions including cavities filled with blood (patient menstruating at the time of the operation). The endometrial tissue in this situation may have been primarily derived from a direct extension downward from that in the cul-de-sac or emboli in the venous circulation of the uterus may have been carried to the vaginal wall through the vaginal veins. I believe that the former is the more likely source.

FIG. 58. Photomicrograph ($\times 25$) of a portion of the section shown in Fig. 57. A cavity is present partially lined by epithelium and filled with blood containing bits of endometrial tissue cast off by the menstrual reaction. Bits of endometrial tissue, End., identical with those in the cavity are present in small veins to the right of this cavity. Some of these fragments lie free in the blood of these veins and others are attached to or implanted on the walls of the vessels (Figs. 59 and 65). What is the origin of the endometrial emboli in these vessels? Are they metastatic from the uterus or were they disseminated from the ectopic endometrial cavities in the vaginal wall? I believe the latter (see Figs. 60 and 61).

FIG. 59. Two photomicrographs ($\times 130$) of portions of the section shown in the preceding illustration. *a* is a longitudinal section of a vein with a small bit of endometrial tissue lying free in its blood, *b* a cross-section of a vein with a strip of epithelium "buckled" in its lumen and *c* a cross-section of a vein distended by endometrial tissue growing in the vessel. For a photomicrograph of another vein containing endometrial tissue see Fig. 66.



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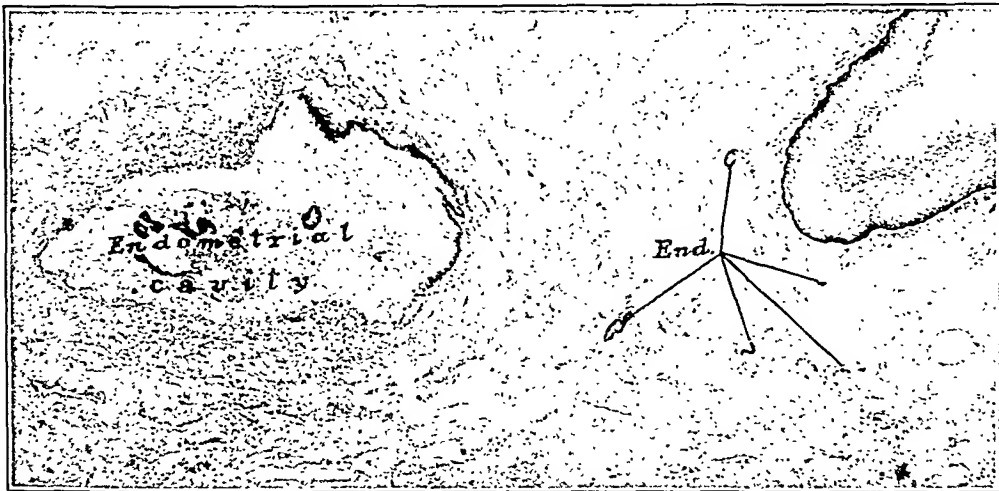
FIG. 60. Photomicrograph ($\times 25$) of a section from the same block as the preceding. Two endometrial cavities are present, the larger just beneath the vaginal mucosa. Both of these cavities are filled with blood (menstrual) containing cast-off bits of endometrial tissue. The smaller cavity has ruptured into a vein at B and some of its contents are escaping into the lumen of the vein. The larger cavity has also ruptured into a vein at A and some of its contents are likewise escaping into the lumen of this vein (see Fig. 61).

FIG. 61. Photomicrograph ($\times 310$) of a portion of the section shown in A of Fig. 60. The endometrial cavity, above, has ruptured through the endothelial lining of the vein and some of the contents of the former have escaped into the lumen of the vein. I believe that the endometrial emboli in the veins of the vaginal wall already shown (Figs. 58 and 59) had a similar origin. As some of these emboli have become implanted on the lining of these vessels (see Figs. 59 and 66) it is again evident that bits of endometrial tissue, cast off by menstruation, are sometimes alive and capable of becoming implanted on the endothelial surface of veins. If bits of endometrial tissue escape into veins during the menstrual reaction of ectopic endometrial tissue in the vaginal wall, we would expect that a similar condition might arise in ectopic endometrial tissue in any situation including a direct or primary endometriosis of the uterus.

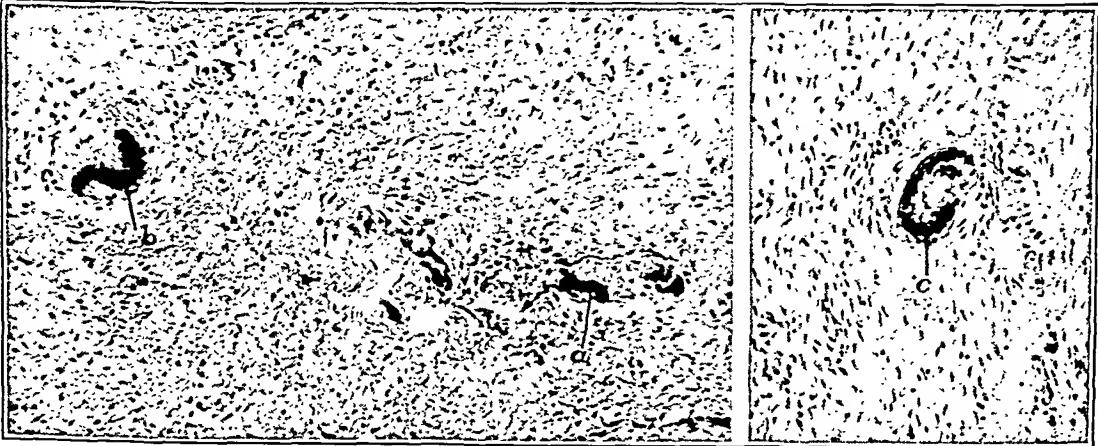
FIG. 62. Photomicrograph ($\times 130$) of a cross-section of a vein in the vaginal wall at a distance from the endometriosis shown in the preceding illustrations. The vein was situated in the lower part of the portion of the vagina which had been excised. A mural thrombus is present containing a bit of "endometrial tissue." We would expect that similar bits of endometrial tissue might escape into the general venous circulation and be carried to the lungs. The patient had as a postoperative complication, "a bronchopneumonia" from which she recovered. Is it possible that this might have been due to a "shower of endometrial tissue" disseminated by the trauma of the operation?



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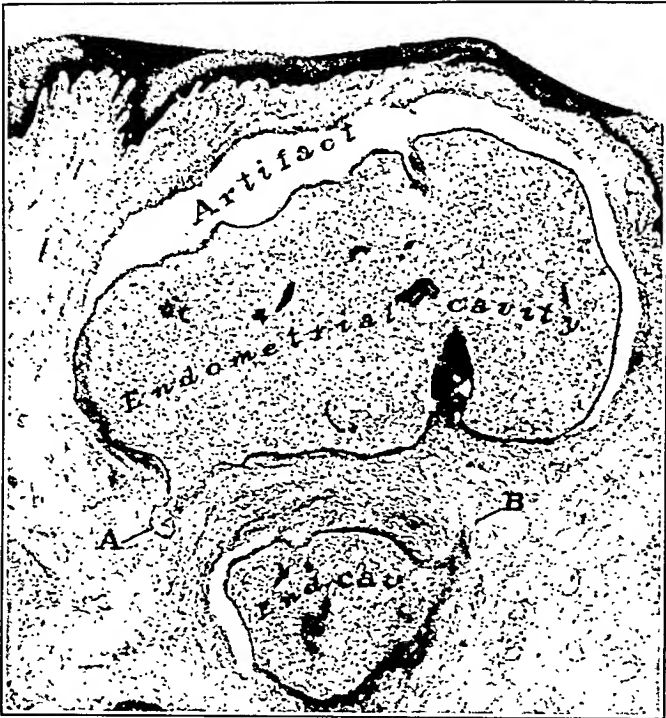


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PLATE 40

FIG. 63. Photomicrograph ($\times 10$) of another section of the vaginal wall again showing many endometrial lesions, including one just beneath and eroding the vaginal epithelium.

FIG. 64. Photomicrograph ($\times 25$) of the vaginal wall from the same block as the preceding illustration demonstrating the erosive action of endometrial tissue on the vaginal epithelium. The endometrial cavity contains blood and bits of endometrial tissue set free by the menstrual reaction. In time the overlying vaginal epithelium might rupture and the contents of the cavity would escape into the vagina just as similar cavities in the ovary or any pelvic structure might rupture and their contents escape into the peritoneal cavity. The hemorrhagic areas of the posterior vaginal wall (see Fig. 67) are due to the accumulation of menstrual blood in these subepithelial endometrial cavities.



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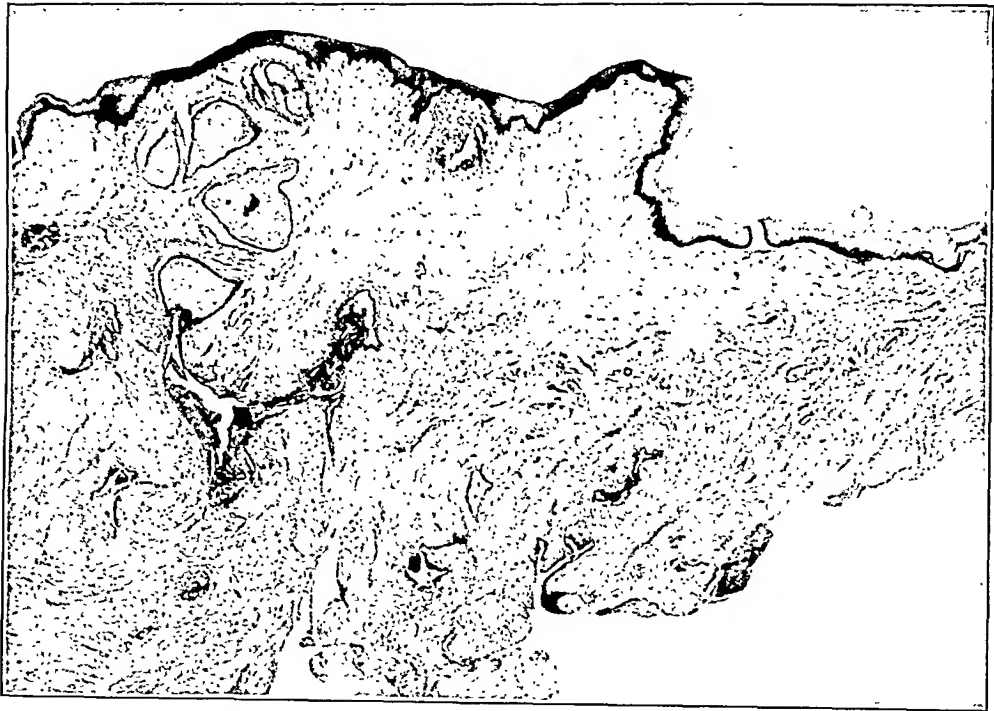
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PLATE 41

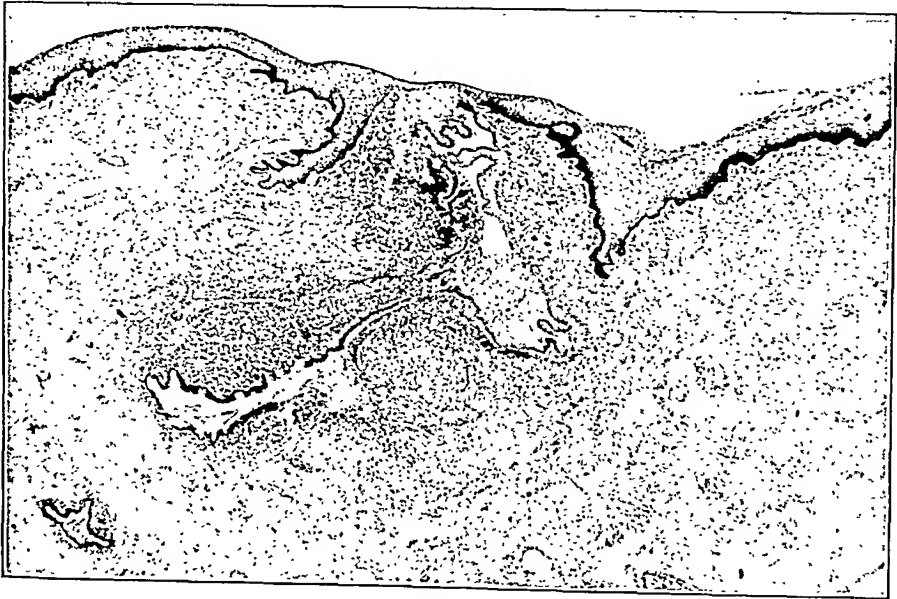
FIG. 65. Colored photomicrograph ($\times 130$) of a portion of the peripheral zone of the right half of the posterior uterine wall (Case 4). The patient was operated upon the second day of menstruation. Fragments of endometrial tissue (emboli) are present in two veins (probably two sections of one vein). It is natural to assume that they were set free by the menstrual reaction of either an embolic growth in a vein or else from the mucosa lining the uterine cavity. The implantation of such emboli in veins would give rise to lesions similar to those shown in Figs. 41, 49 and 52.

FIG. 66. Colored photomicrograph ($\times 130$) of a portion of the posterior vaginal wall (Case 4) showing endometrial epithelium implanted in a vein, one of the veins indicated in Fig. 58 (see also Figs. 59, 60 and 61).

FIG. 67. Cervix and portion of the posterior vaginal wall (natural size) showing the characteristic appearance of the endometriosis in this situation. The hemorrhagic elevations are due to the accumulation of menstrual blood in subepithelial endometrial cavities (see Figs. 63 and 64).



63



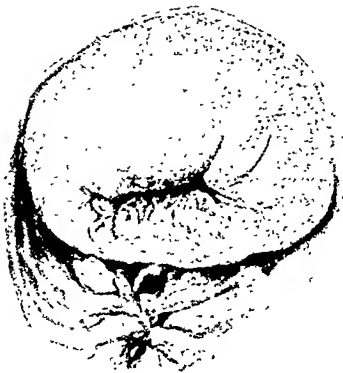
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of which there is also blood. Vascular channels connecting the arterioles with the venous sinuses are left to the imagination of the student of such a specimen. Yet it must be evident that along these finest channels the most important activities of the spleen are accomplished.

TECHNIC

The human spleens used in this study have been obtained in part as fresh surgical material from patients subjected to splenectomy for various reasons and in part from early necropsies. The surgical specimens usually presented distinct abnormalities but offered the advantage of excellent fixation in the freshest possible state. The spleens from necropsy were removed from one to several hours after death, but in many instances presented no recognizable pathologic changes. In either case the procedure followed in the laboratory has been essentially the same. After rapid gross description, including weight and measurements, consistence and external appearance, one sears the capsule at one end and, by puncturing with a sterile glass capillary pipette, removes a sample of pulp for aerobic and anaerobic bacteriologic culture. One next cuts off a portion of the organ at this end, placing thin slices in various fixing fluids for histologic study in the undistended state and also preparing smear preparations of the pulp which may be variously stained in the search for special types of cells or parasites. Next, by blunt dissection of the fat at the hilum, one isolates an arterial branch which enters near the intact end of the organ and ties into this vessel a suitable glass cannula. Through this are injected at moderate pressure perfusion fluids, first Locke's gelatin solution (sodium chloride 9.2 gm., sodium bicarbonate 0.05 gm., potassium chloride 0.1 gm., calcium chloride 0.15 gm., gelatine 2.5 gm., water 1000 cc.), then a small amount of physiologic salt solution and finally Helly's fixing solution (potassium bichromate 2.5 gm., sodium sulphate 1 gm., mercuric chloride 5 gm., water 100 cc., formalin 5 cc.) until at least a small portion of the spleen substance shows the yellow discoloration due to this fluid. The artery is then clamped and the cannula removed. Next one inserts a larger cannula into one of the venous branches coming from near the middle of the organ and carries out a venous perfusion in a similar manner. It is sometimes well to ligate lightly the severed end of the spleen to prevent the too rapid loss of fluid in the venous

THE FINER VASCULAR CHANNELS OF THE SPLEEN *

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INTRODUCTION

Among those organs and tissues of the body of which the essential anatomy and physiology, in both health and disease, still remains more or less obscure, the spleen is conspicuous as an organ of large size about whose finer structure considerable uncertainty still remains. Keen students of the structure of the spleen have not been lacking. As one reads the contributions of Gray,¹ of Mall² and, above all, of Weidenreich,³ he gains the impression that the subject is already quite fully elucidated. Unfortunately the subsequent publications of Helly,⁴ of Mollier⁵ and the recent contribution of Thoma⁶ have called into question Weidenreich's description of the finest vascular channels of the spleen. The anatomy of the circulation in this organ is of fundamental importance to all other considerations which may concern its physiology, its pathologic anatomy or its abnormal function. Weidenreich found that most of the arterial capillaries of the spleen open out into spaces of the spleen pulp so that the blood circulation is an open one. Helly and, more recently, Thoma oppose this view absolutely and conclude that the arterial capillaries open directly into the venous sinuses and that the blood circulation is a closed system similar in this respect to the blood circulation of other organs and tissues. The still more recent paper of Robinson⁷ lends support to the advocates of the open circulation.

A brief study of the ordinary sections of spleen as they have usually been prepared for histologic study will quickly show that the finer structure of this organ cannot be recognized in such specimens. One can distinguish follicles and pulp, arterioles near the center of the follicles and occasionally in the pulp he may distinguish a venous sinus filled with blood and outlined by pulp cords in the substance

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THE ARTERIAL CAPILLARIES IN SERIAL SECTIONS

The arterial capillaries of the spleen may be classed for purpose of description into three groups: (1) follicular capillaries, (2) capillaries of marginal zone and (3) capillaries of the pulp cords.

The follicular capillaries come off in considerable numbers from the follicular artery and pass almost straight through the substance of the follicle to its periphery, where they pass only a short distance beyond the capsule. Some of these vessels evidently anastomose by means of short communicating branches at the level of the capsule. The terminal twigs extend into the less compact marginal zone where they terminate by numerous small openings into the spaces of the spleen pulp of this marginal zone, always at considerable distances from the nearest venous sinuses. The endothelial cells lining the terminal portion are branched and their processes are in continuity with similar processes of the pulp cells proper. Evidently the state of the terminal endothelium is subject to variation. Sometimes the openings into the pulp appear large and permit ready escape of corpuscles. At other times the openings appear to be constricted by the contraction of the endothelial cells so that erythrocytes are held back and even the passage of liquid is impeded. In such cases the terminal portion may be distended in the form of an olive or an acorn to present the picture of an ampulla. These capillaries evidently provide a rich nourishment for the follicles. The plasma passes through the capillary wall as lymph to bathe the reticulum of the follicle and the closely packed proliferating cells in the meshes of this reticulum. It is probable that dissolved substances, including toxic agents, escape with this fluid and come into direct relation with the lymphocytes and reticular cells of the follicle. Relatively few blood cells escape from the vessels here. On this account, the terminations of these vessels at the marginal zone contain blood excessively rich in formed elements and poor in plasma. These formed elements have to escape through minute openings between the branches of endothelial cells into the pulp spaces. They thus come into very intimate contact with the terminal capillary endothelium and with the immediately continuous reticulo-endothelial cells of the pulp, which is loosely arranged with wide meshes in the rather broad marginal zone about the follicle. Abnormal blood cells, especially those which have become somewhat adhesive through injury, are readily phagocytized

perfusion. A considerable portion of the organ should remain visibly distended. After ligating the vessels the entire mass is immersed in Helly's solution for four to six hours, then divided into slices, the portion perfused through the artery being placed in one bottle, the vein-perfusion slices in another and portions of the remaining substance in a third. One is thus provided with a variety of material from the same spleen for study in sections. The subsequent handling presents nothing out of the ordinary except that the mercury must be removed and the serial paraffin sections must be cut at a thickness of 4 microns or preferably 3 microns. A firm paraffin should be used so that portions are not displaced by the knife in cutting. Excellent technic is essential here.

The study of human material has been supplemented by the study of animal spleens from rabbits, guinea-pigs and dogs, most of the material being from rabbits. Here it has been possible to use not only normal organs but also to utilize the spleens of animals in which distinct pathologic alterations in the spleen have been experimentally induced; furthermore, to employ intravital injection of foreign substances such as nucleated erythrocytes from birds and suspensions of opsonized bacteria as well as to perfuse the spleen with the fixing fluids during the life of the animal. Such injections of the living spleen are best done by inserting a very fine glass cannula into one of the short gastric branches of the splenic artery and injecting the fluid through this back into the main artery from which it passes to the spleen by the force of the blood pressure of the animal.

The cannula is made from ordinary soft glass tubing by drawing out one end to a very fine capillary and fusing on a short side-arm near this tip. The capillary tip has to be smoothed in the flame with great care so that the edges of the minute tip will not too readily cut the vessel wall. This smoothing is accomplished by heating the tip in the gas flame while blowing a current of air through the lumen to prevent sealing of the tip. When properly smoothed, the glass tip shows rounded free edges when examined under the low power of the microscope. Its roughness may also be tested by scratching the surface of the finger nail. The lumen at the tip should not be narrowed. Some practice may be required before one is successful in making serviceable cannulas.

into the pulp without evident ampulla. Occasionally, also, one can trace such a pulp capillary as a definite vessel quite to the wall of a venous sinus into which it opens by an extremely narrow cleft between endothelial cells. This type of termination, first clearly recognized by Weidenreich, appears to be the exception rather than the rule, even in the termination of the pulp cord capillaries. The size of the opening is small and ordinarily of the same order as the stomas in the wall of a venous sinus; it is by no means similar in structure or in functional capacity to the capillary-vein union in other organs. These pulp-cord capillaries are formed of a single layer of endothelial cells with nuclei projecting into the lumen. External to this wall is a sheath of spleen pulp similar to that of all the pulp cords, consisting of branched reticular cells enclosing pulp spaces which lead over to venous sinuses on either side. Evidently much of the plasma escapes from the lumen of the pulp-cord capillary in its course so that concentrated corpuscles come to the termination of it. Capillaries of this group although rather conspicuous and more easily traced than those of the first two groups, are much less numerous and evidently far less significant for the function of the spleen.

The above anatomic description, based upon the study of serial sections of distended human and rabbit spleens, differs little from the description of Weidenreich. The method is open to certain legitimate criticisms which have been previously voiced in regard to studies of this kind. First, distension of the spleen by perfusion through the vessels may rupture the more delicate structures and produce artificial openings and passages where naturally none exists. This criticism has been urged against all conclusions based upon anatomic injections of the spleen. The point of this criticism is largely lost, however, when the perfusion is carried out by introducing the perfusion fluid through a branch of the splenic artery in the living animal and permitting it to be forced into the spleen at the arterial pressure. A second criticism appears at first somewhat more serious, namely, that in very thin serial sections of the distended spleen the individual elements, cells, nuclei and fibers are irregularly displaced by the knife of the microtome, so that one is not able to recognize the successive portions of the same structure in successive sections. In our series of approximately a hundred spleens there are excellent illustrations of this difficulty. Only when the perfusion fixation has succeeded well and the dehydration and embedding and the micro-

by the endothelial and pulp cells at this place. Those blood cells which pass this searching inspection escape from the pulp through the abundant stomas of Mollier by which the pulp spaces freely communicate with the lumina of the venous sinuses.

The capillaries of the marginal zone are given off from arterial branches which run out from the follicle into the pulp. Such a branch gives off from three to ten divisions which quickly terminate in the substance of the marginal zone about the follicle and one or two much longer terminal capillaries which extend peripherally into a pulp cord between venous sinuses. The centripetal twigs are usually much curved and elusive in serial sections. They are rather short and do not anastomose. They often branch dichotomously just before terminating and the very tip is usually distended to a thin-walled ampulla from which many narrow and irregular clefts lead into the pulp spaces of the marginal zone. The axis of the terminal ampulla is nearly tangential to the capsule of the follicle. These vessels are somewhat larger and more readily distended than are the follicular capillaries. Except for the terminal portion their walls are conspicuously thick, consisting of a layer of rather large endothelial cells with nuclei standing out in the lumen. This endothelium is surrounded by a syncytium of rather large and compactly arranged cells which also appear to be endothelial in type but with minute lymph spaces between them. These may be in several layers and by their more compact arrangement are conspicuous in the less compact pulp of the marginal zone and pulp cords. They constitute the arterial sheathes of Schweigger-Seidel.⁸

The capillaries of the pulp cords are derived from the same trunk as the preceding group. They are more easily followed in serial sections of the distended spleen, because they often run fairly straight or curve chiefly in one plane so that three to five sections may sometimes suffice to trace the course of such a capillary from its origin by final branching of its trunk vessel to its terminal extremity in the pulp between the venous sinuses at a distance from the follicles. In the distended spleen such a vessel is wide enough to contain a single row of erythrocytes. Its termination presents some variations. In some instances there is an olive-shaped terminal ampulla twice as wide as the capillary proper and outlined by strands of endothelial cells between which numerous clefts permit communication with the adjacent pulp spaces. In other instances the vessel opens directly

showing abundant bird's corpuscles caught in the pulp meshes, especially in the marginal zones, a few bird's corpuscles passing through the stomas in the sinus walls and very few such corpuscles within the sinus lumen (Fig. 16). Evidently the corpuscles in the stomas are in transit *from the pulp spaces to the sinus lumen*.⁹

The results of these experiments confirm in a satisfactory manner the interpretation which has been reached by the study of the serial sections.

SUMMARY

1. There is proposed a technic for the fixation and preservation of human spleens obtained by surgical splenectomy or at necropsy, which makes possible a more illuminating study of the structure of this organ.

2. For the study of animal spleens a modified perfusion technic is described, in which the perfusion fluid or experimental injection material is introduced into a short gastric branch of the splenic artery.

3. The blood circulation in the spleen is essentially an open circulation in that the pulp spaces constitute the connecting channels through which the blood passes from arterial capillary to venous sinus, a mechanism which brings about intimate contact of the blood plasma with the cellular elements of the follicle and a similar intimate contact between the formed elements in the blood and the reticulo-endothelial cells of the marginal zone and the pulp cord.

4. It is believed that an adequate understanding of the splenic circulation and the application to normal and pathologic material of the methods employed here may be of service in advancing our knowledge concerning the normal physiology, the pathologic anatomy and the abnormal function of this rather enigmatic organ.

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some technic have been quite perfect can one expect to obtain useful serial sections of spleen at a thickness of 3 microns. We do feel, however, that this difficulty has been surmounted in the technical handling of some of our material and we regard the illustrations submitted herewith as justification for this opinion.

EXPERIMENTAL DEMONSTRATION OF THE OPEN CAPILLARY CIRCULATION OF THE SPLEEN

Weidenreich appears to have sensed the weakness of unsupported morphologic evidence and he therefore undertook to furnish experimental evidence as well. He injected the blood of a bird into the ear vein of a rabbit, waited several minutes, killed the rabbit and prepared sections of the spleen. He found the nucleated erythrocytes of the bird in the pulp spaces and concluded that they had reached this situation from the open ends of arterial capillaries. Helly who repeated these experiments reached a quite contrary conclusion. He believed that the bird's erythrocytes passed from the arterial capillaries into the venous sinuses and from here through the clefts in the sinus walls into the pulp. The demonstration by Mollier of the abundant stomas in the sinus walls has been accepted by some modern authors as strong support for Helly's interpretation. There can be no doubt that bird's erythrocytes can be found in such preparations actually half way through the wall of a venous sinus, caught in transit by the fixation, passing, according to Weidenreich, from the pulp spaces to the sinus lumen, or, according to Helly, from the sinus lumen to the pulp space. It seemed to us that this question might be elucidated by fixing the spleen at a very much shorter interval after introducing the foreign corpuscles. Therefore, instead of injecting them into the ear vein, we introduced the foreign corpuscles into the splenic artery through one of its short gastric branches in the living animal and then followed this injection by perfusion with salt solution and fixing fluid (Helly's formalized Zenker solution) within one minute. We hoped by such technic to be able to find the bulk of the nucleated erythrocytes either in the sinuses with few or none in the pulp if Helly's view should be correct, or on the other hand, if Weidenreich were right, to find them abundant in the pulp and few in the sinuses. After a few trials to perfect the technic it was possible to obtain quite convincing preparations

first one indistinct and without lumen in this section, extending upward a little to the left of the larger trunk. The second branch, just to the left of this, shows a distinct lumen for a short way but then passes out of the section. The third twig continues downward and to the left to terminate in a distended ampulla which is rather indistinct. Other capillary branches extend to the right in the picture, most of them terminating in the marginal zone about the follicle seen at the bottom of the picture.

FIG. 4 (Photomicrograph). — Centripetal arterial capillaries of the marginal zone in the human spleen, from same spleen as Fig. 3; section stained with phloxine-azure B. The thick Schweigger-Seidel sheath suddenly fades out as the vessel enters the marginal zone of the follicle where it divides and terminates in two ampullae, the upper one being distinct in this section. This photograph was shown before the Albany meeting of the American Association of Pathologists and Bacteriologists on April 3, 1926. The colored drawing of Fig. 14 represents a part of the same field.

PLATE 44

FIG. 5 (Photomicrograph). — Arterial capillaries of the pulp cords in rabbit's spleen, distended and fixed in the living animal. The follicle with its dilated artery is seen at the right. Arterial capillaries, venous sinuses and pulp cords are easily distinguished. Hematoxylin-eosin stain.

FIG. 6 (Photomicrograph). — Capillary of the pulp cord in rabbit's spleen, almost complete in a single section. Hematoxylin-eosin stain.

PLATE 45

FIG. 7 (Photomicrograph). — Section next in series to that of Fig. 6, showing termination of the same pulp capillary, with a well defined minute channel leading from the dilated ampulla into the adjacent venous sinus, the channel blocked by an endothelial cell which is about to escape from it into the venous sinus.

FIG. 8 (Camera-lucida drawing). — The same ampulla shown in Fig. 7, showing more clearly the detail of the rod-like endothelial cells lining the terminal arterial capillary, the pulp reticulum, a large phagocyte with ingested erythrocyte at lower right corner and, somewhat indistinctly, the wall of a venous sinus. The connection between ampulla and venous sinus is extremely narrow and blocked by an endothelial cell which has ingested an erythrocyte. The channel cannot be found in adjacent sections of the series. Length of the scale line represents 50 microns.

PLATE 46

FIG. 9 (Photomicrograph). — Arterial capillary of pulp cord of human spleen; from same spleen as Fig. 3; section stained with phloxine-azure B. The capillary is cut longitudinally for a long distance. In adjacent sections it was easily traced back to an arteriole with thick Schweigger-Seidel sheath. At its termination there is slight dilatation of the lumen to form an ampulla and the pulp spaces at either side are greatly distended. It does not open into a venous sinus. This photograph was shown at the Albany meeting of the American Association of Pathologists and Bacteriologists on April 3, 1926. The ampulla and adjacent structures are shown in colored drawing in Fig. 15.

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DESCRIPTION OF PLATES

PLATE 42

- FIG. 1 (Photomicrograph). — Capillary vessels of the splenic follicle of the rabbit. The living spleen was perfused with Locke's solution followed by Helly's fluid to fix it in the distended state. The excentric follicular artery remains widely dilated and several thin capillaries are visible radiating from near the center of the follicle. Some of the latter show a distinct lumen. (Hematoxylin-eosin stain.)
- FIG. 2 (Photomicrograph). — Termination of a follicular capillary at margin of follicle in human spleen. The patient was a child of eight years presenting the manifestations of purpura with a marked platelet deficiency, for which splenectomy was performed. This section is from the portion distended by arterial perfusion stained with phloxine-azure B. At the center of the picture the end of a follicular capillary opens out like a fan and its mural endothelium becomes continuous with the pulp reticulum of the marginal zone. This same capillary termination is shown in a drawing in Fig. 13.

PLATE 43

- FIG. 3 (Photomicrograph). — Arterial capillaries of the marginal zone in rabbit's spleen, distended and fixed in the living animal. The section has been stained with hematoxylin-eosin. The largest dilated vessel at the top is surrounded by a somewhat indistinct Schweigger-Seidel sheath. The capillaries below it come off as a single trunk in the third section away from this one. The open capillary extending to the left presents three branches, the

PLATE 49

FIG. 15 (Camera-lucida drawing). — The identical ampulla of pulp capillary of human spleen shown in Fig. 9. Detail of the capillary wall, of pulp reticulum and of wall of venous sinuses is somewhat more distinct here than in the photomicrograph (compare Fig. 10). Adjacent sections in the series reveal no direct pathway from ampulla to venous sinus. Length of scale line represents 50 microns.

FIG. 16 (Camera-lucida drawing). — Follicle, marginal zone and adjacent pulp of rabbit's spleen into which a suspension of bird's erythrocytes was introduced by injection into short gastric branch of splenic artery during life, followed one minute later by perfusion with Locke's solution and Helly's fixing solution to produce prompt fixation in the distended state. Note that the nucleated erythrocytes are abundant in the pulp spaces of the marginal zone and are escaping thence into the venous sinuses through the stomas of Mollier. This drawing and the microscopic field represented by it were both demonstrated before the American Association of Pathologists and Bacteriologists and the International Association of Medical Museums at Albany, New York, on April 3, 1926. (F) follicle; (FA) follicular artery filled with erythrocytes of rabbit; (MZ) marginal zone; (P) pulp cord; (Ph) phagocytic cell; (V) venous sinus; (1) avian erythrocyte passing through sinus wall from pulp of marginal zone; (2) avian erythrocyte sharply constricted at its middle in passage through wall of venous sinus. Length of scale line represents 100 microns.

PLATE 47

FIG. 10 (Camera-lucida drawing). — Arterial capillary of pulp cord in spleen of rabbit; serial sections at 3 microns, stained with hematoxylin-eosin; first of three sections in series. (1) nucleus of endothelial cell appearing also in the next section, designated to permit ready orientation; (2) nucleus of endothelial rod cell forming part of the wall of the terminal ampulla of arterial capillary, also appearing in part in the next section; (3) phagocytic pulp cell, also in the next section; (4) large nucleus recognizable also in the following section; (AC) arterial capillary; (DIA) diapedesis of nucleated wandering cell; (F) floor of a venous sinus; (P) pulp; (V) venous sinus. Length of the scale line represents 50 microns.

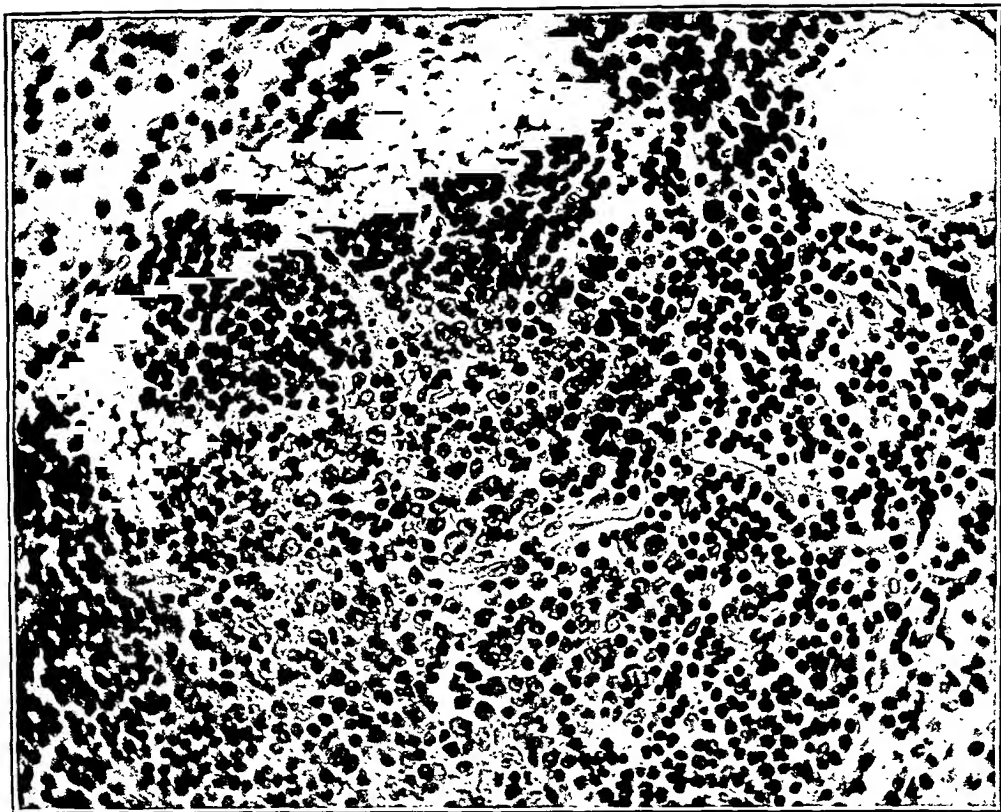
FIG. 11 (Camera-lucida drawing). — Section next in series below that of Fig. 10. The terminal ampulla of the arterial capillary is almost complete in this section. (1) (2) (3) (4) nuclei facilitating orientation with Fig. 10; (11) nucleus appearing also in the next section, designated to assist orientation; (AC) arterial capillary; (F) floor of venous sinus; (P) pulp; (Ph) phagocytic cell; (V) venous sinus. Length of the scale line represents 50 microns.

PLATE 48

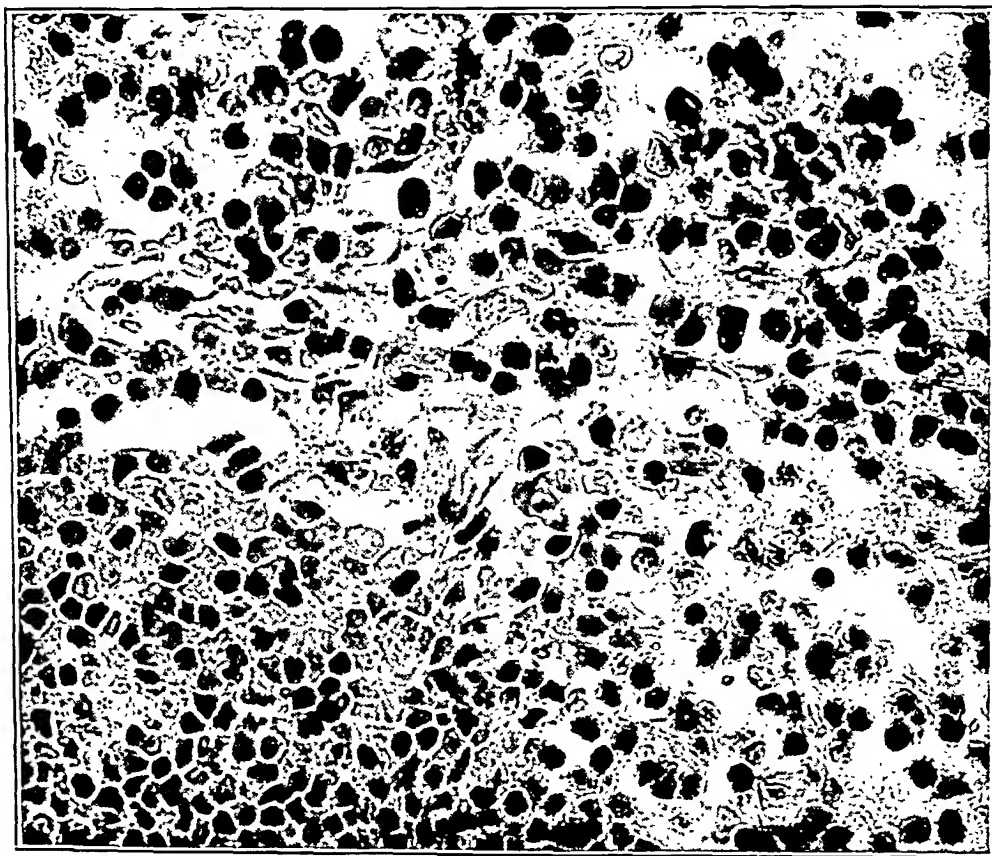
FIG. 12 (Camera-lucida drawing). — Third section of the series, next below that of Fig. 11. The site of the terminal ampulla is here hardly distinguished from ordinary pulp reticulum. (11) nucleus for orientation with preceding section, Fig. 11; (AC) arterial capillary; (DIA) wandering cell in diapedesis; (P) pulp; (Ph) phagocyte; (R) roof of venous sinus appearing for the first time; (V) venous sinus. In this series of three sections enough has been included in the drawings to indicate a distinct layer of pulp all about the terminal ampulla separating it from neighboring venous sinuses. The fenestrated character of the wall of the ampulla and of the walls of the venous sinuses is distinctly evident. Length of scale line represents 50 microns.

FIG. 13 (Camera-lucida drawing). — The identical terminal ampulla of follicular capillary of human spleen shown in Fig. 2. The drawing shows more distinctly the narrow lumen, the slender rod-shaped endothelial cells of the capillary wall going over by insensible gradations to the reticular pulp cells of the marginal zone. In adjacent sections of the series one can trace this vessel back to the center of the follicle. Length of scale line represents 50 microns.

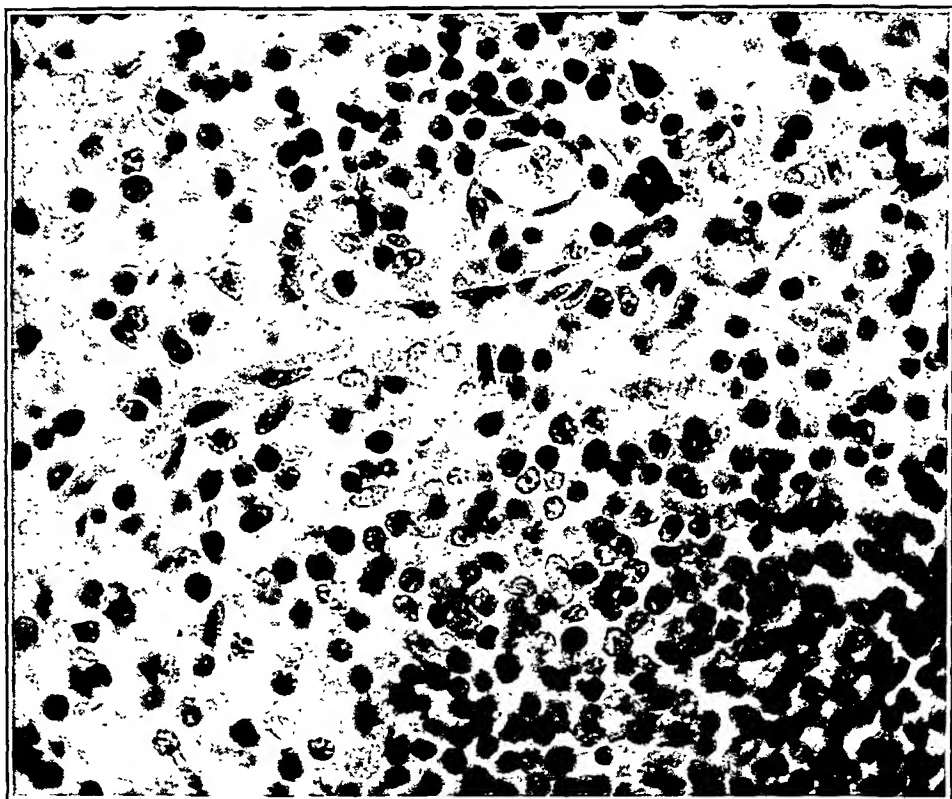
FIG. 14 (Camera-lucida drawing). — The identical terminal capillaries of marginal zone of human spleen shown in Fig. 4, where the relation to spleen follicle can be ascertained. The detail of capillary wall and of pulp reticulum is more distinct in this drawing. The upper ampulla is directly continuous with the capillary lumen. The lower capillary is more irregular at its termination and what appears to be ampulla here is seen, on closer study, to be a dilatation located in the pulp at one side of the capillary termination. It would appear that the variable state of contraction of the mural endothelium at the moment of perfusion may be responsible for variations of this sort. Length of scale line represents 50 microns.



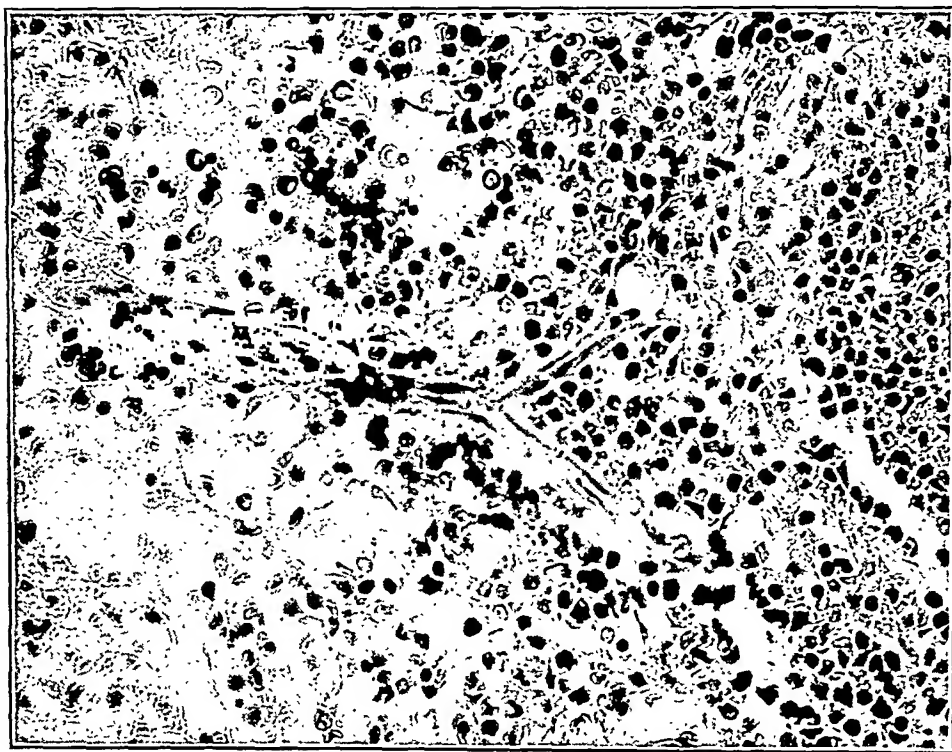
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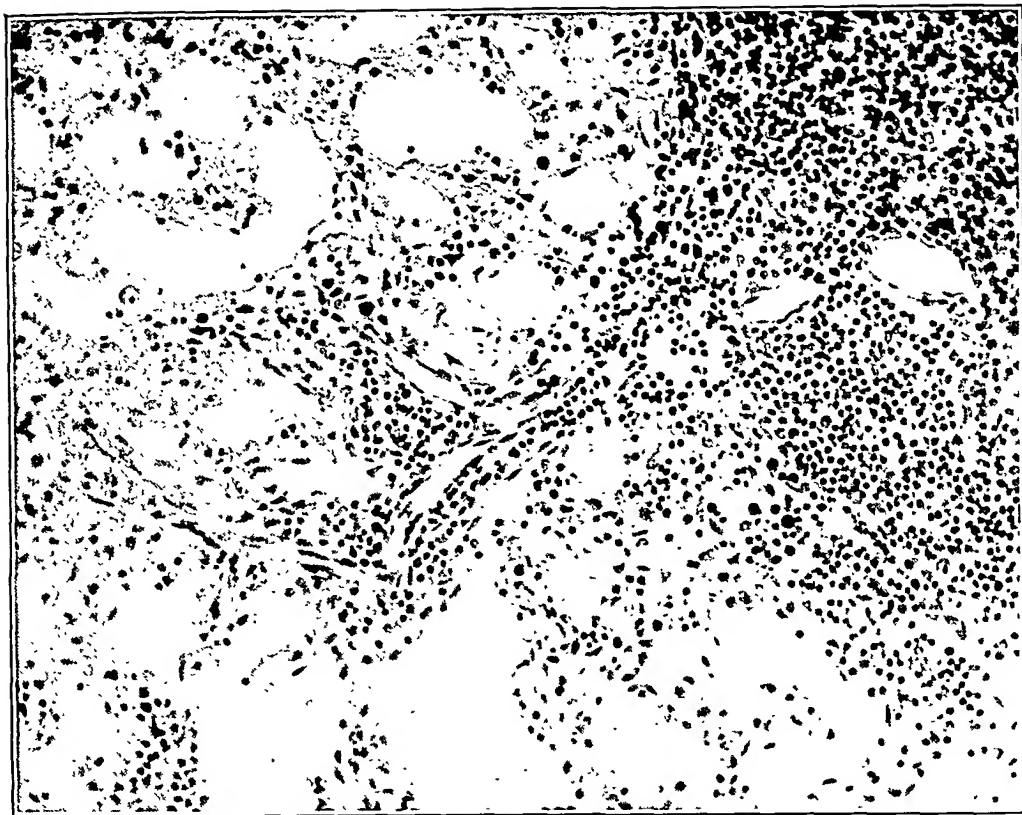
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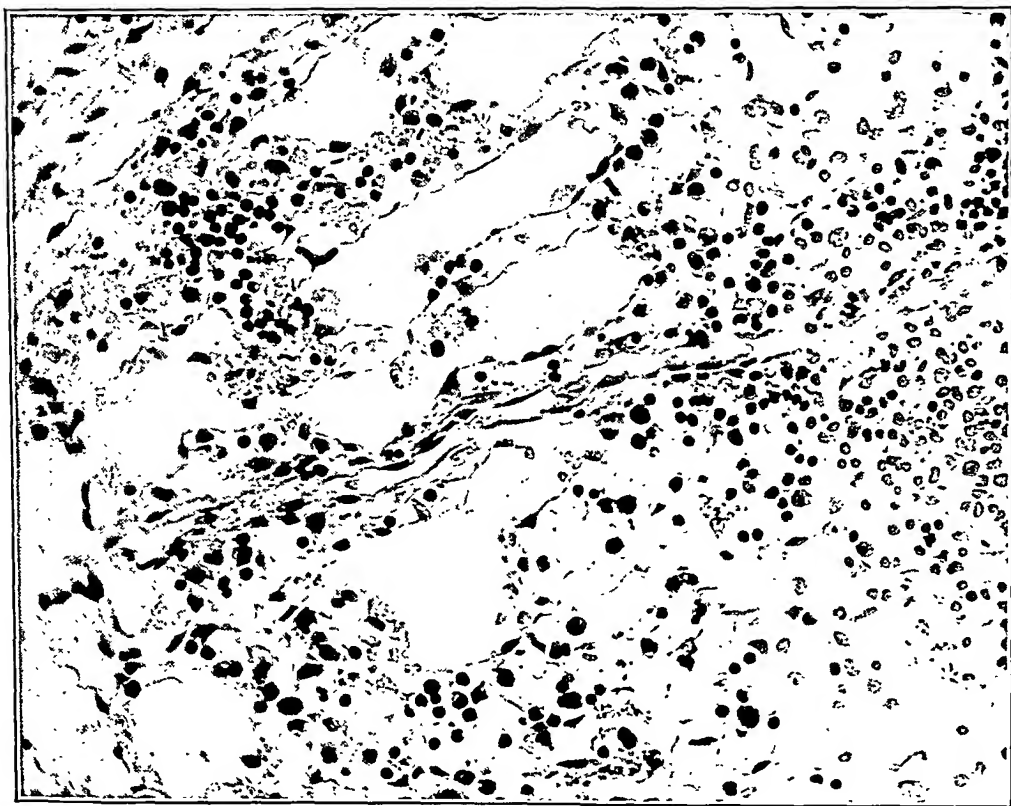
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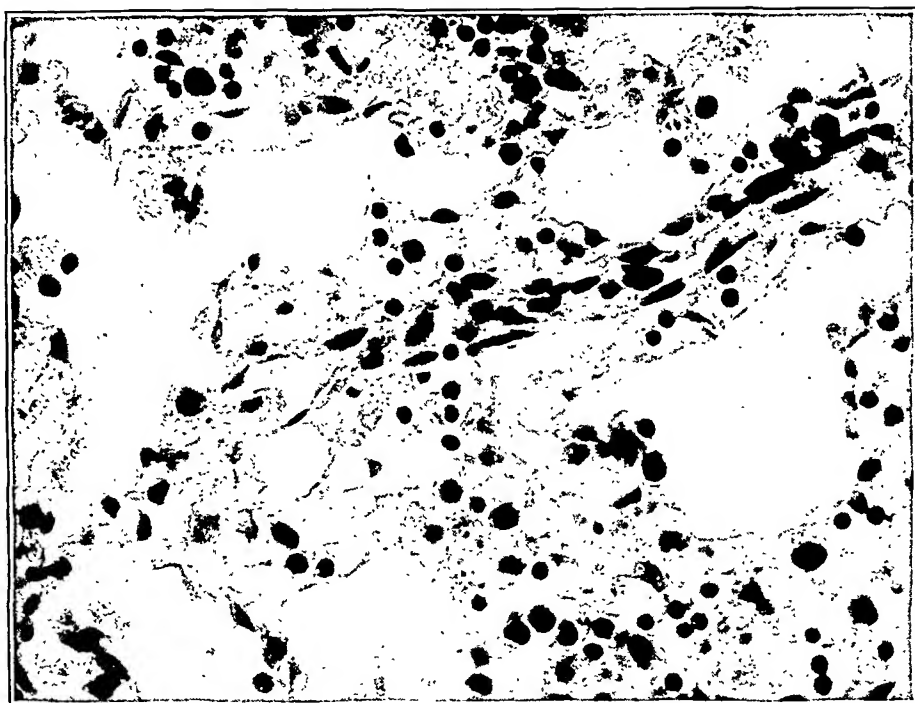
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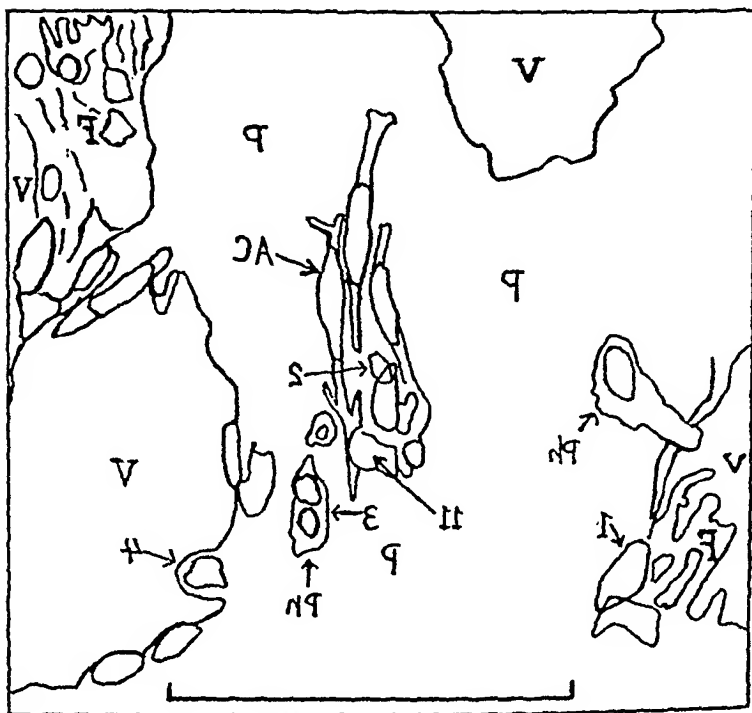
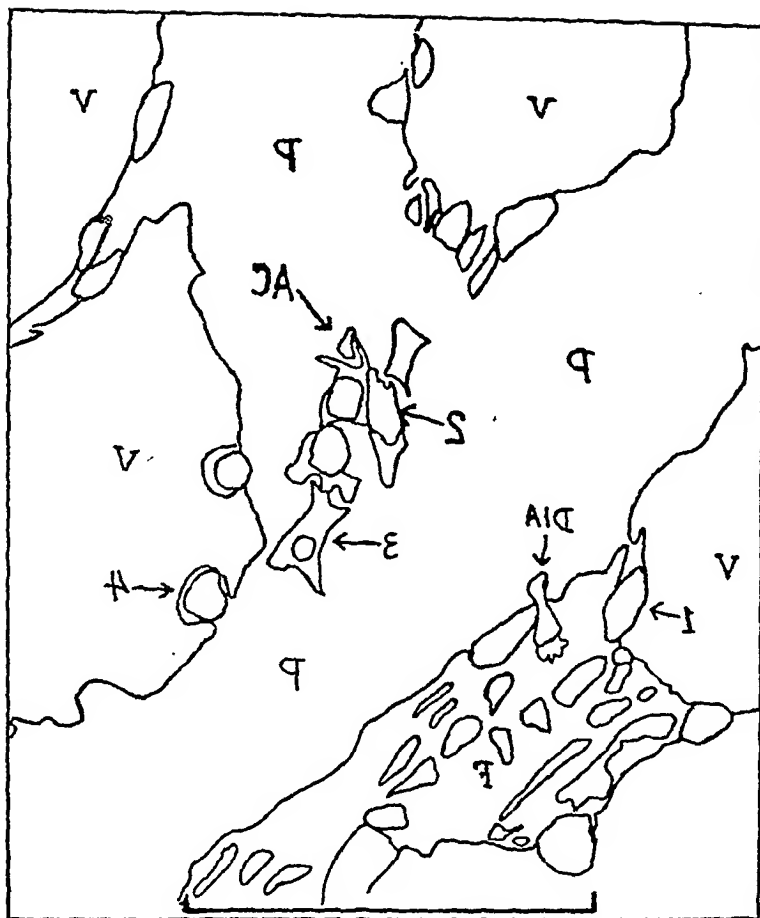


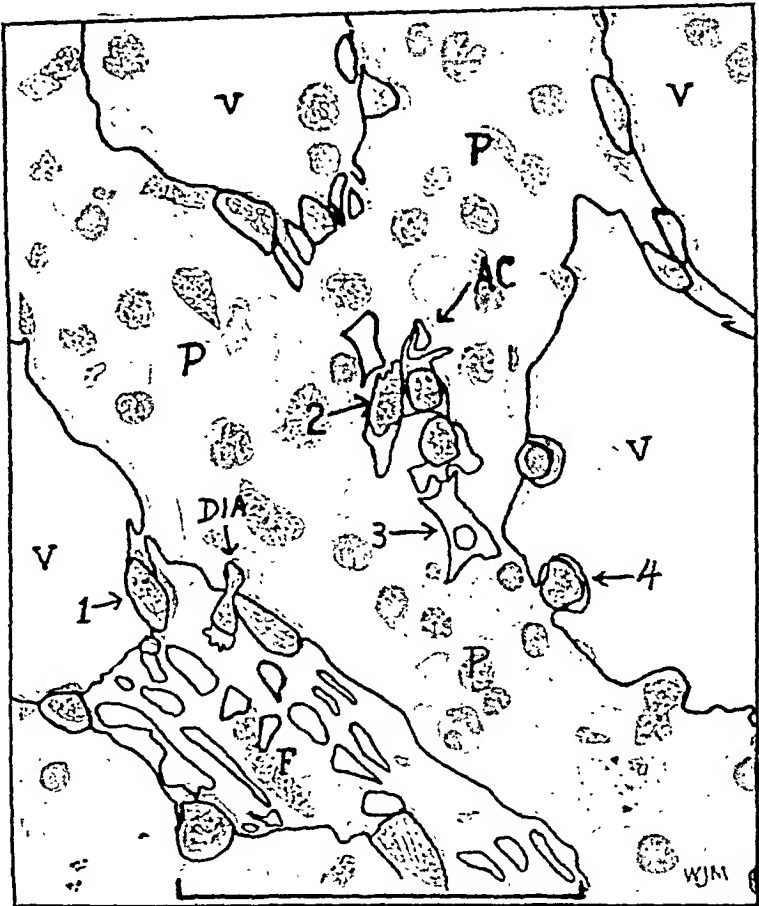
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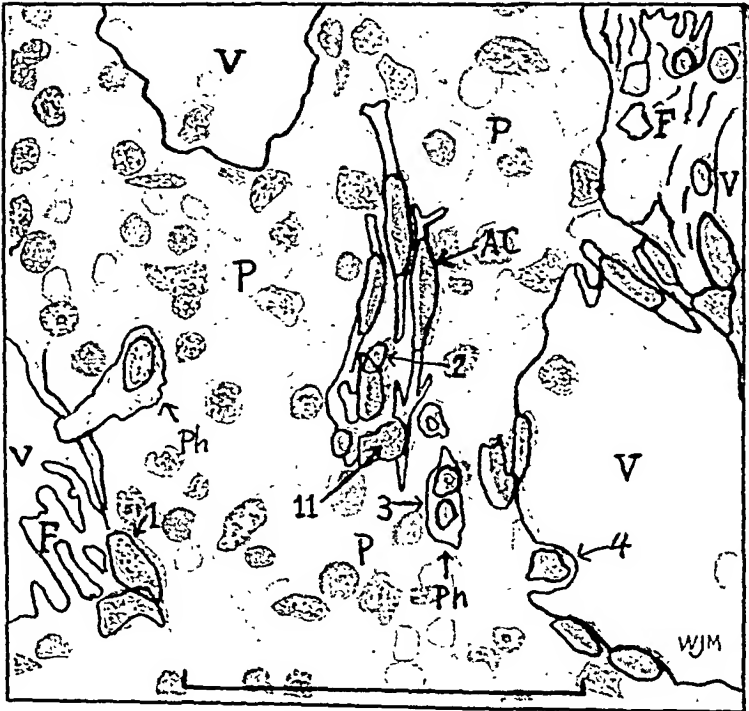
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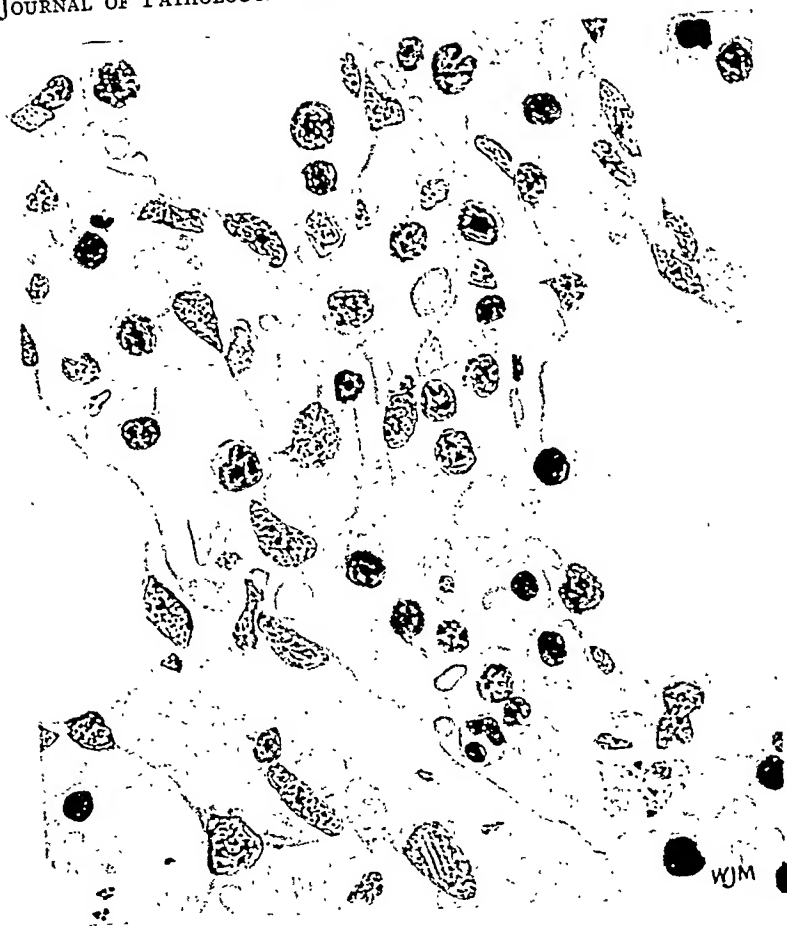




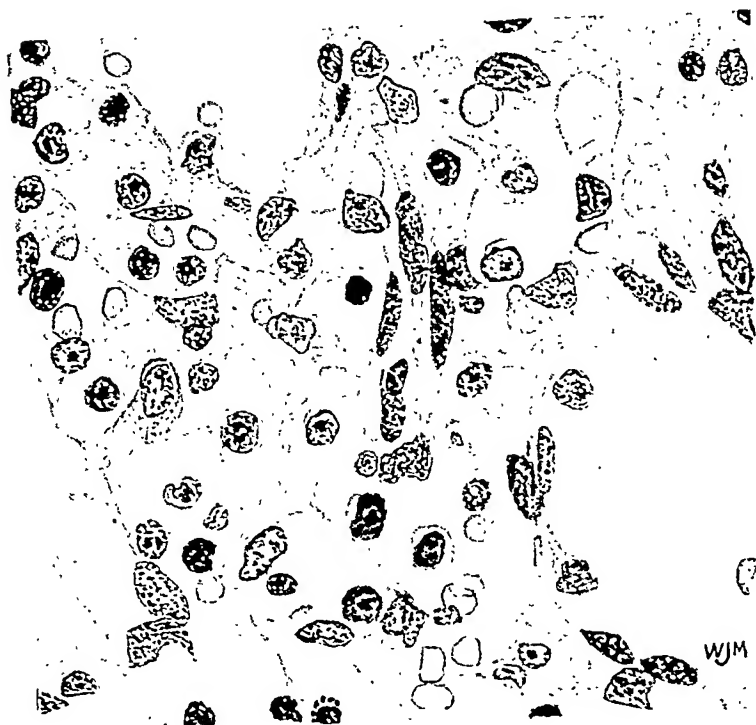
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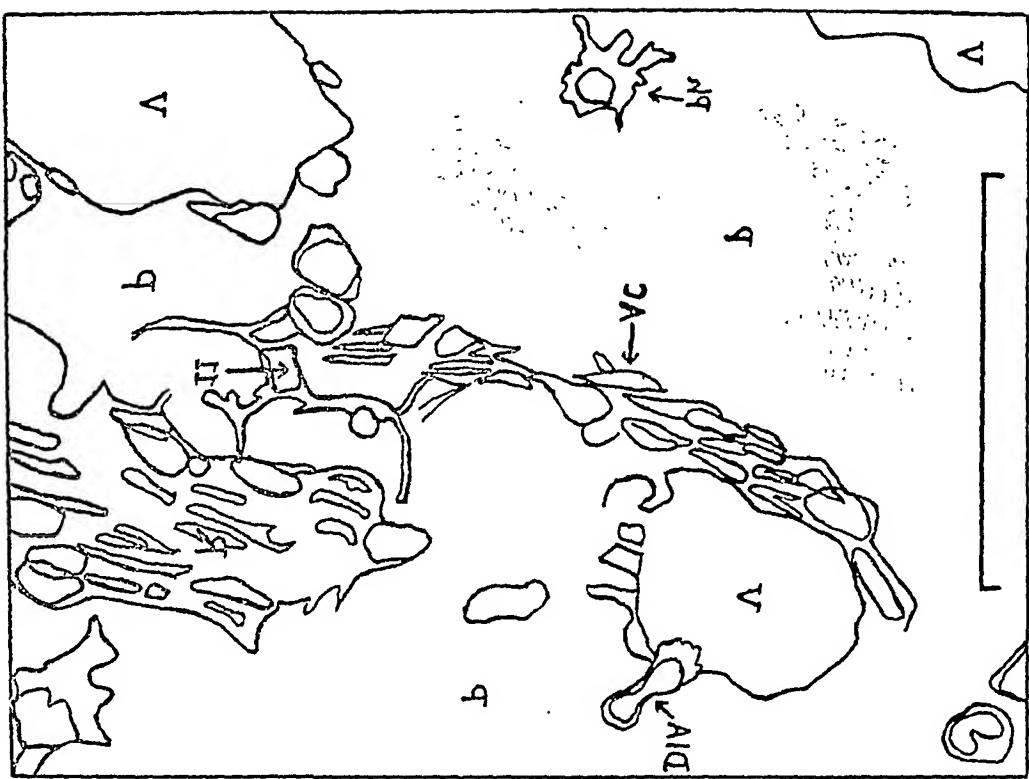
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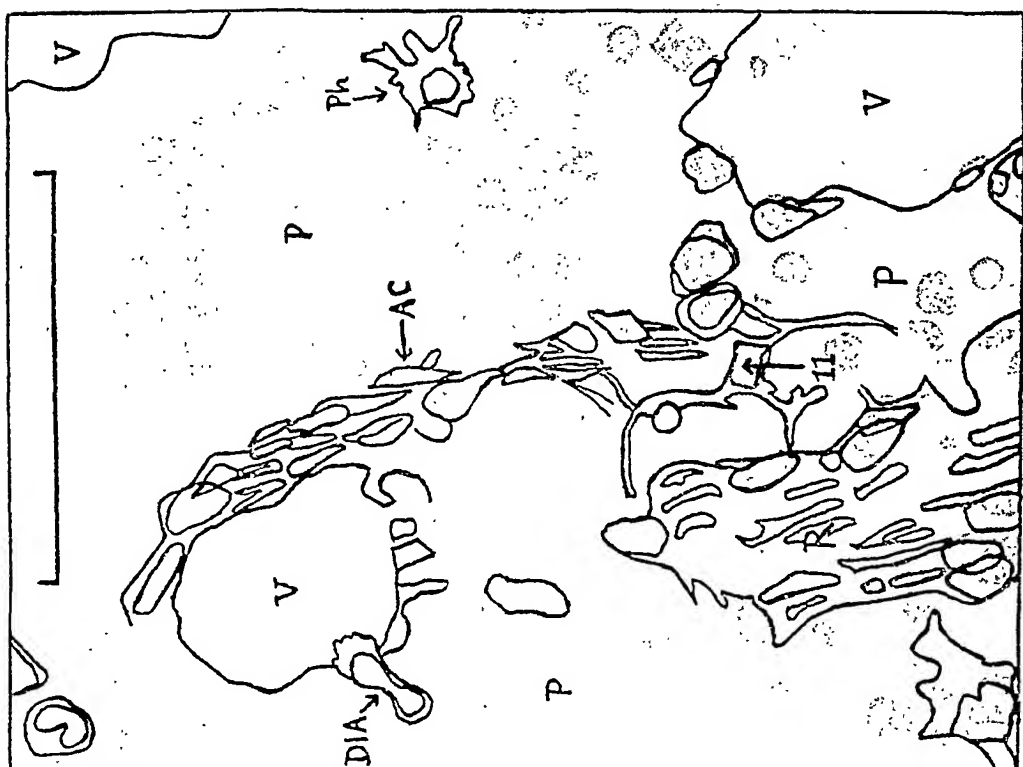


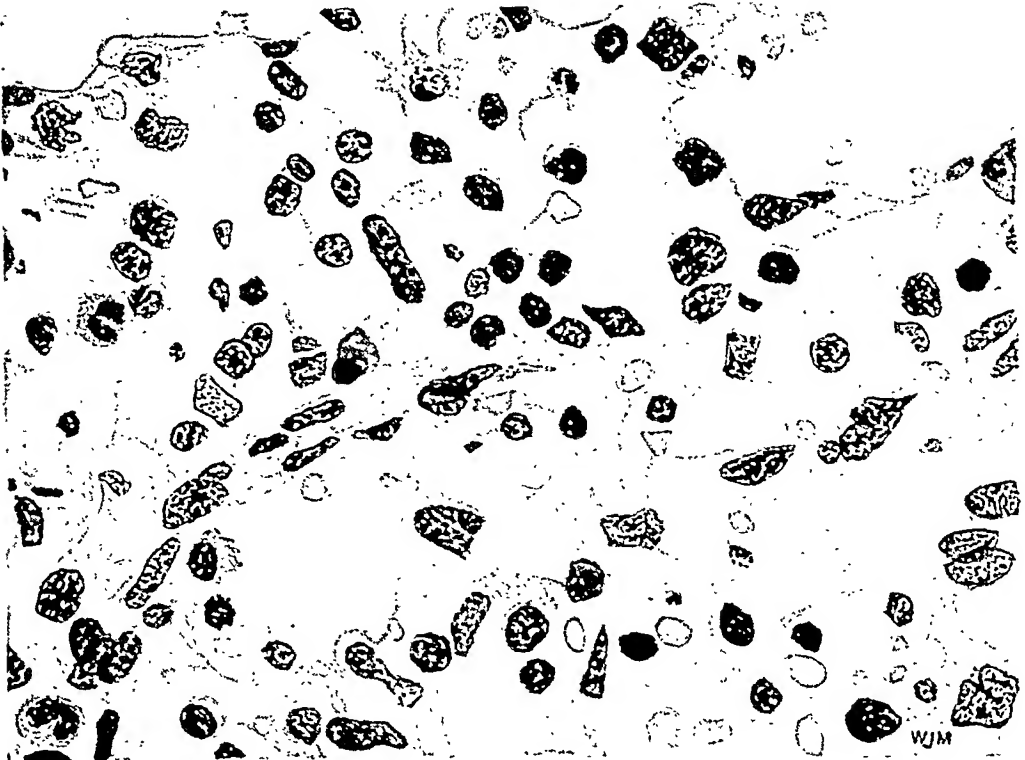
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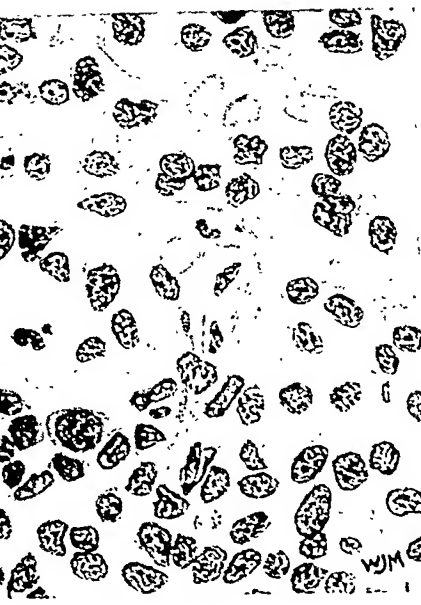
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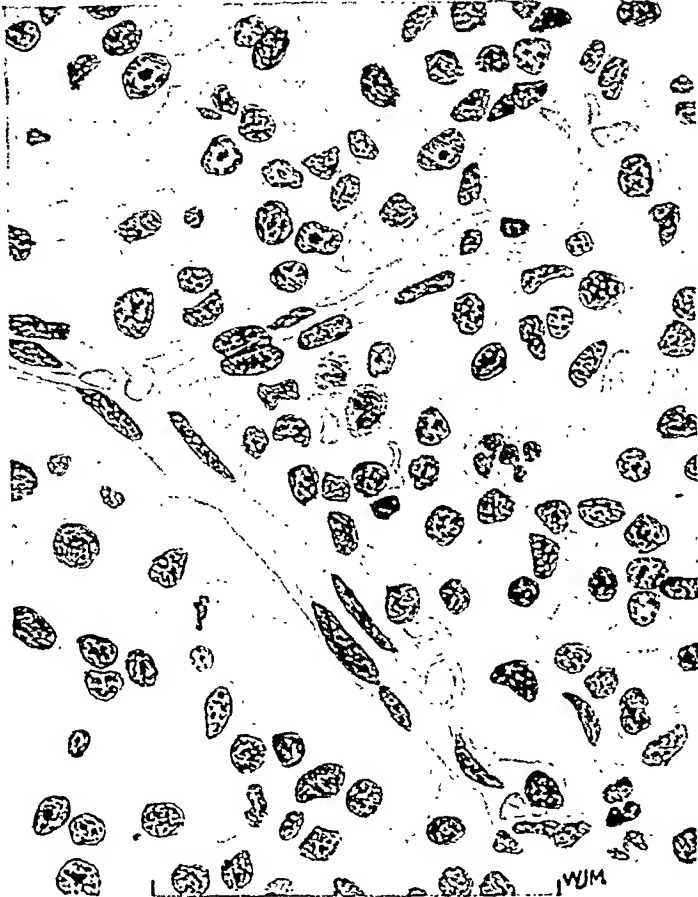




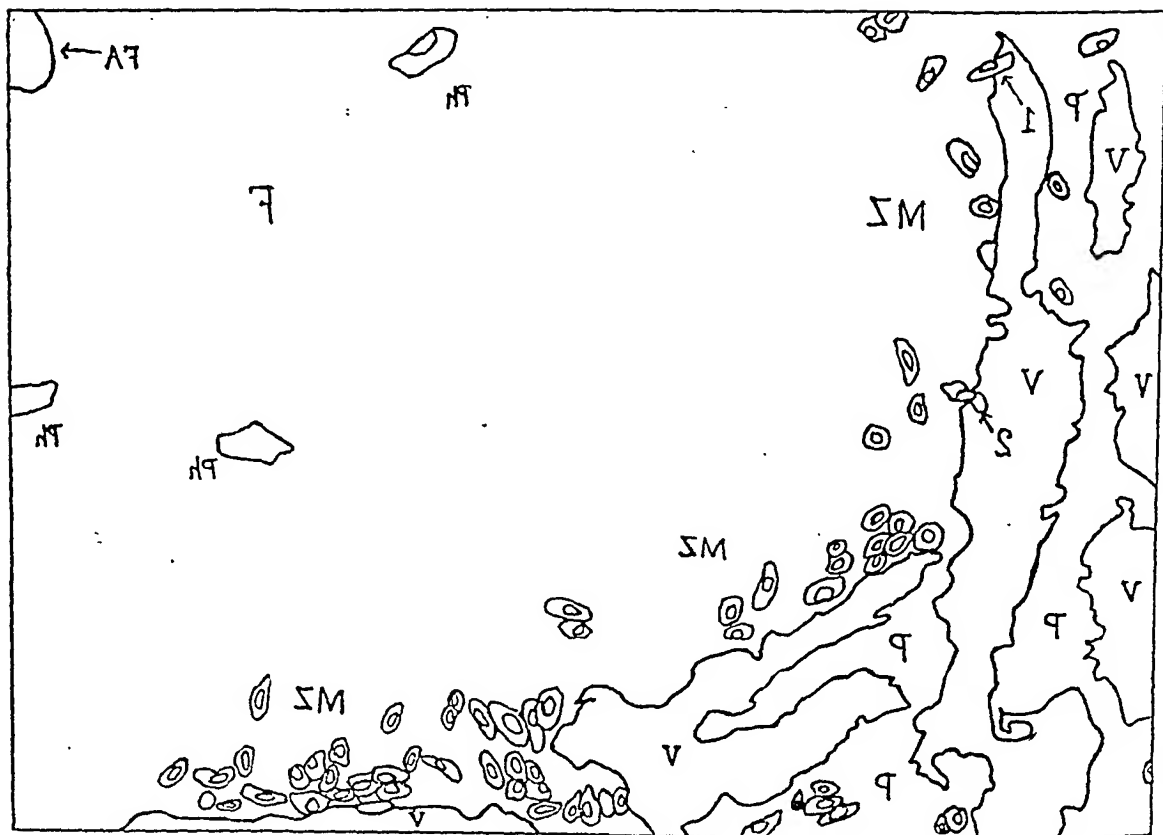
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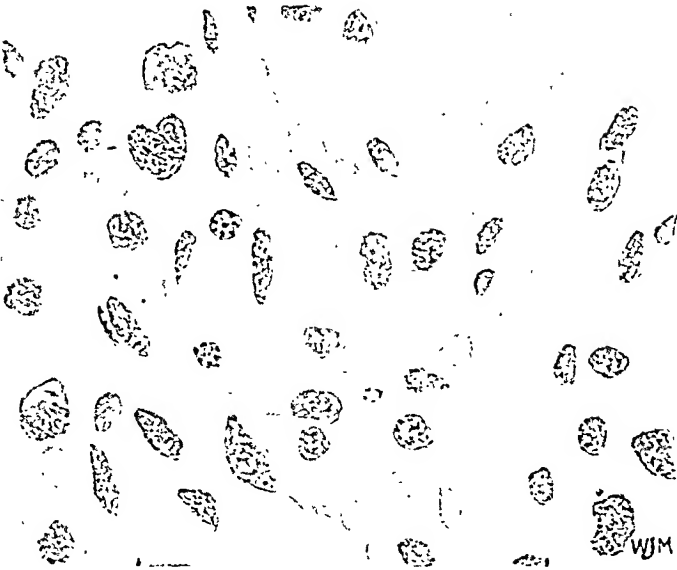


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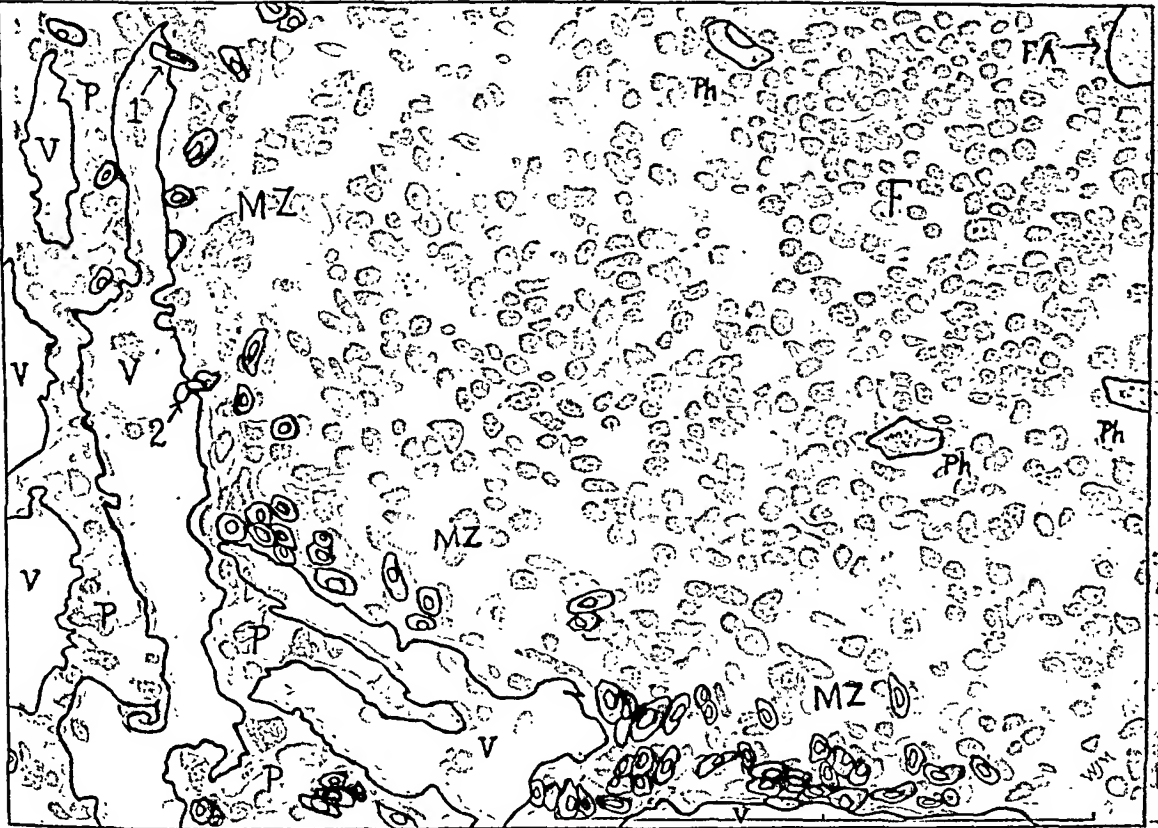


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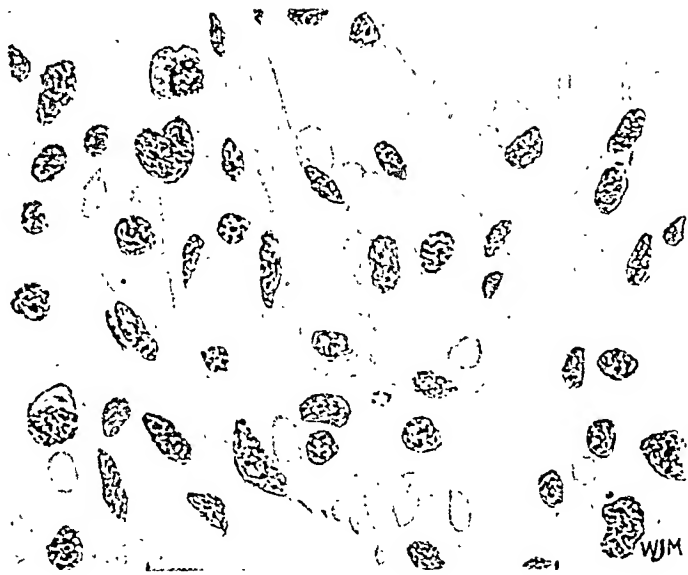




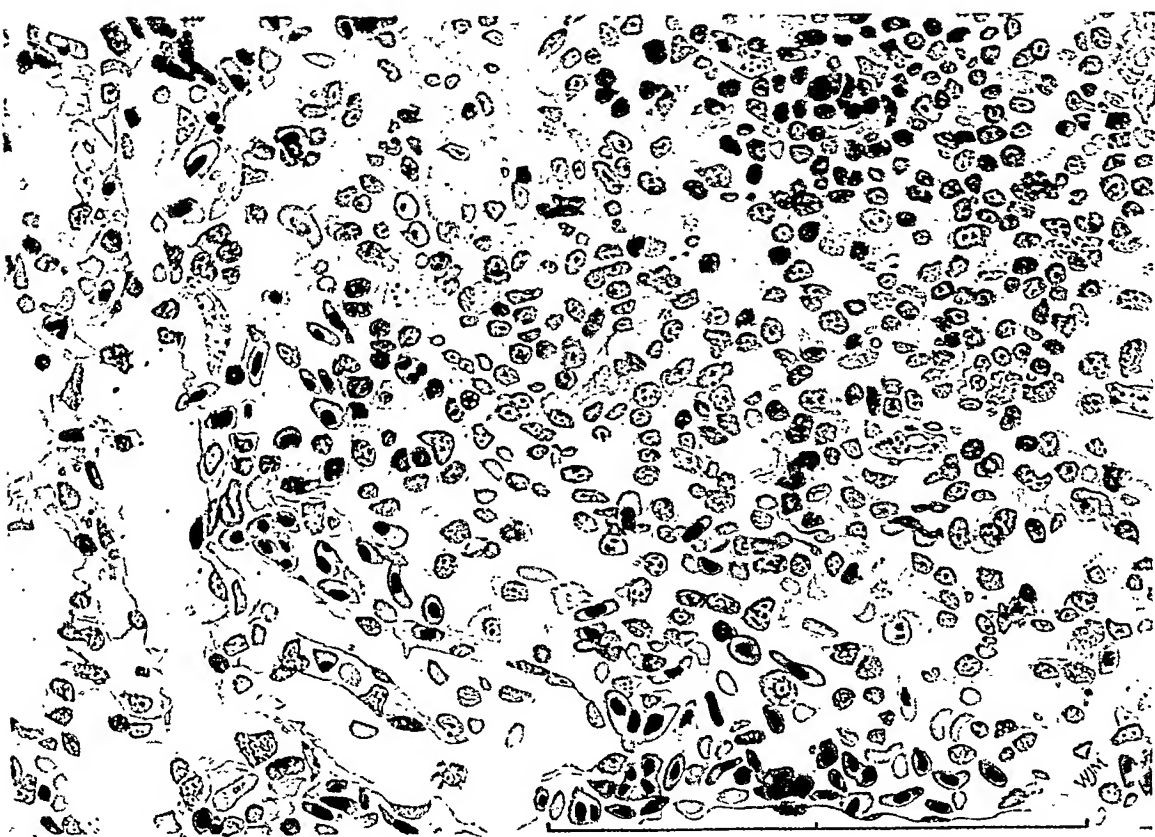
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in the pancreas of infants. Even in the same section there can be recognized islands of exceedingly great variety in size. Therefore, if one finds an occasional large island among the other ordinary ones in a certain pancreas, one cannot maintain that this condition represents a hypertrophy of the islands.

If one finds, however, great numbers of unusually large islands in a pancreas, one may consider them enlarged. Koch,⁹ Lang,¹¹ Cecil² and Warren²⁵ have reported cases described by them as adenoma of the islands. Nuboer,¹⁴ Martius,¹² Hertel⁶ and Gray⁵ have also described cases of hypertrophy. Seyfarth¹⁹ described a case of hypertrophy in the pancreas of a child which died of inanition.

We have also observed a pancreas in which very large islands were found throughout the organ, while other specimens showed exclusively small islands.

Accordingly, there is no doubt that a process of hypertrophy of the islands may occur under certain conditions. However, whether such enlargement does occur by transition of the acinar cell or by hyperplasia of the island cell proper, is still under discussion in the literature. We believe that knowledge of the histogenesis of the enlargement may contribute to the decision of this question. Therefore, we shall first discuss this point in our attempt to come nearer to the solution of this problem.

The enlarged islands generally show the following characteristic features:

(a) They are exceedingly irregular in size and show no complete limiting fibers around the islands; they usually are connected with the acini at the greater part of their circumference. (Fig. 1.)

(b) In the periphery of the island between the insular and acinar cell groups there are found fine fibers having no relation to the capillaries in the islands, which by their location, however, appear to be remnants of the basement membrane of the acini. (Figs. 1 and 2.)

Fig. 1 shows one example of these relations; we believe that this island is in a process of hypertrophy. The fine fibers are located especially at the periphery of the island and in the center there are rather thick fibers enclosing capillaries, which apparently were present in the island before its enlargement. The fine fibers are derived from the basement membrane of acini, which is proved by serial sections and also by the fact that they have no relation to the capillaries within the island tissue.

STUDIES ON THE ISLANDS OF LANGERHANS IN THE HUMAN PANCREAS*

II. SIGNIFICANCE OF VARIATIONS IN STRUCTURE

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In a previous paper,¹⁶ we have concluded that the islands vary in structure: some islands are connected with acini or ducts, while others are separated from the rest of the pancreatic tissue. After having established this morphologic fact we now intend to discuss the significance of these variations.

I. ISLANDS CONNECTED WITH ACINI

That the majority of the islands are connected with acini without intervening fibers has been clearly demonstrated in our previous paper. However, whether such connection really means a transition from acinus to island or *vice versa*, as interpreted by Laguesse,¹⁰ Gellè,⁴ Vincent and Thompson,²³ Seyfarth¹⁹ and others must be considered more carefully, because even Weichselbaum and Kyrle²⁴ and Opie,¹⁵ who believe in an anatomic independence of the islands, admit an occasional break in the limiting fibers around the islands; so did Bensley¹ express his view quite emphatically that such connection does not necessarily mean transition between the two cell groups, though he assumes that the majority of the islands are in direct contact with the acini. Since Bensley's work has been done with the greatest care, many investigators accept his opinion. In the most recent literature Macleod¹³ maintains that acini and islands are as distinct and separated from each other, both anatomically and physiologically, as are the anterior and posterior lobes of the pituitary glands.

As a matter of fact, no sufficient proof has as yet been offered by any of the previous workers which would settle the question of the anatomic relation of the islands to the acini.

If one examines a great many specimens of pancreas, occasionally one can find islands of unusually large size; such islands also are seen

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ducts and terminal ducts. From its location in this large island we believe that this glandular structure is in a stage of conversion from acinar cells to island cells.

It is common in the adult pancreas to find peculiar altered cell structures within the acini which stain very dark with hematoxylin and are spindle-shaped (Fig. 6). The same cells are found within the very large islands which evidently are in a process of enlargement, especially at the periphery. Figs. 6 and 7 represent typical examples.

Fig. 7 is a section from a pancreas which shows a great number of unusually large islands in every portion. This picture was made from the periphery of a very large island, where such dark cells differing from capillary cells are seen.

Fig. 8 shows an irregularly shaped island. In the lower portion there is an insular structure indicating the original island, the other light cell groups representing newly formed island structures which can be interpreted as being limited by the basement membrane of the acini on account of the presence of the acinar cells between the light cells. Some of these basement membranes contain capillaries. This picture as well as Fig. 7 indicates the histogenesis of capillary formation in the growing islands. The basement membranes of the acinar cell groups, which contain a capillary, remain in the enlarging island as part of its capillary system.

The fact that peculiar homogeneous cells appear often among the acinar structures in the adult pancreas has been described by Opie,¹⁵ Weichselbaum and Kyrle,²⁴ and Fahr.³ Opie described these cell groups as altered acini, the lumen being usually very conspicuous, often dilated. We observed, however, the homogeneous cell groups without any remarkable dilatations of their lumen and frequent hyperplasia of these cell elements. Fig. 9 shows a group of these homogeneous cells.

Fig. 10 shows the same cell group with a mitotic figure of a centroacinar cell. An acinar cell has lost its basophilic staining reaction and apparently represents a transitional stage between the acinar and homogeneous cells.

Fig. 11 demonstrates the homogeneous cell in groups. Among these a few acinar cells are still present. Though no distinct cell division figure can be seen in this section, there undoubtedly exists a process of hyperplasia of homogeneous cells. In this pancreas numerous large islands are found.

(c) At the boundary of these islands there are found various changes in cell elements; these are observed in the hematoxylin-eosin preparations (Figs. 3 and 4). The cell groups which apparently belong to the acini, stain neither with hematoxylin nor with eosin distinctly. They do not show basophile or zymogen granules. They contain central nuclei but they are different from the insular cells. In short it cannot be pointed out to which cell group they belong.

These findings are more clearly demonstrated in the preparations stained with neutral gentian violet; in the same acinus two cell groups can be found which differ from each other. Besides there is a third type which does not stain with this dye distinctly.

These three findings alone already suggest that the enlargement of the islands occurs mainly by transformation of acinar cells into island cells, not alone by hyperplasia of island cells proper, because mitosis of insular cells within large islands has been found very rarely; therefore, we may infer that such occurrence is not necessarily a characteristic feature of enlarging islands. Naturally our negative result would not necessarily exclude the possibility of a hyperplasia of island cells. However, we can advance other arguments against such a view.

If enlargement occurs by hyperplasia of the insular cells only, we should be able to observe evidence of pressure by the peripheral cell groups of the very large islands on the neighboring acinar tissue. These findings have never been observed in our material. These relations are demonstrated in Figs. 1 and 5. Furthermore, sometimes we have seen a form of large island which consists of a dumb-bell-like fusion of two islands (Fig. 5). The enlarged islands often show unusual irregularity; they protrude into the acinar structures like the arm of an octopus (Fig. 1 and also see Fig. 14 in the previous paper¹⁶). We would be unable to interpret these findings if we accept the enlargement as being caused by hyperplasia of insular cells only.

Fig. 6 shows the periphery of a very large island. In the center there is a peculiar glandular structure consisting of island cells. Its lumen contains a fluid which can be recognized as identical with that in the ducts, because it stains with eosin very deeply and in the next serial section stained with neutral gentian violet, this fluid shows a light brown color, while the blood in the arteries is deep brown. The sections from this pancreas show markedly dilated

situated, suggesting proliferated centroacinar cells. Their location and our previous observations presented in Figs. 10 and 11 support this explanation.

In silver preparations which were kept in 2 per cent silver nitrate solution over 100 hours in the incubator, fine brown or deep brown granules occur in the island cells, while the acinar cells remain clear (Fig. 16). The same granules, however, also occur in a small number of acinar cells, as well as in the duct cells which have no relation to the island; they appear isolated between acinar cells or duct cells (Figs. 16 and 17). These argentophilic granules within the acinar cells have been observed in the frog pancreas by Saguchi.²⁰ We have also found similar granulated cells within acini in preparations stained by neutral gentian violet. This brings us into accord with the opinion of Vincent and Thompson²³ and others who state that insular cells are occasionally scattered within acinar structures. These findings are an indirect argument in favor of transformation of acini to island cells.

After demonstrating on the basis of our morphologic studies that acinar cells are able to change into insular cells, it is logical to concede that the enlargement of the islands takes place by a process of transition of the acinar cells. The interpretations of Opie,¹⁵ Weichselbaum and Kyrle,²⁴ Helly,⁷ Bensley,¹ Ukai²² and others who claim that such does not happen, cannot refute our conclusions, because they never as yet have given such clear-cut findings as ours and moreover our conclusions are based upon the findings, described in our first paper, that most of the islands are connected with acini, without intervening fibers.

Whether the reverse may be possible, namely, changing of island cells into acinar structure, as maintained by Laguesse,¹⁰ Seyfarth¹⁹ and others, is difficult to confirm. Certain facts, however, indicate its occurrence. Among numerous specimens we have found specimens of pancreas which contain small islands throughout (Fig. 18), whereas others showed very large ones (see Fig. 14 in first paper¹⁶). In the former type of pancreas the numerous islands still show direct connections with acini and the border of both cell types represents a very gradual transition without any discernible signs of degenerative changes in the island cells. Such pictures could be interpreted as transition from island to acinar cells. The fact that the islands actually present varying structures as described in our first paper,

Fig. 12 illustrates the fact that the homogeneous cell groups may also occur at the periphery of the very large islands. In Fig. 13 one sees several homogeneous cell groups which differ somewhat from the picture described above. They show various stages of transition from acinar to homogeneous cells.

Fig. 14 shows a similar picture. Many of the homogeneous cells stain with eosin very intensely. Among these there are several very dark cells deeply stained with hematoxylin, which often can be found in the acinar structures. This picture also shows various stages of transformation of the homogeneous cells. The structure of this cell group is almost identical with that of an island; however, it cannot be classed as a true island on account of a small number of dark cells surrounding the homogeneous cell groups which appear to be acinar cells by their morphologic characteristics. Inasmuch as this island-like cell group is bordered by rather thick connective tissue, which is proved by serial sections, these dark cells do not belong to the neighboring acini.

The appearance of the cell groups, presented in Figs. 9 and 10, is not exactly identical with that of the island, although it resembles the latter in general makeup. Figs. 13 and 14 come even nearer to the composition of the island. On account of the presence of hyperplastic processes among these homogeneous cell groups and also because at present we cannot distinguish the homogeneous cells from those of the islands, it is reasonable to assume that these cells represent a certain stage in the transformation of acinar cells into island cells, *i. e.*, that they are the actual island-builders. Their occurrence at the periphery of very large islands and also in pancreases that contain numerous very large islands supports this view strongly.

The island cells are composed of so-called A and B cells whose granules stained by Lane's method are distinct from those of the acinar cell. This fact was advanced as an argument, especially by Bensley, Macleod and others, in favor of the different structure of the two cell groups, and led to their conclusion that no transition is possible from one to the other. However, these granules (A and B) are not present in every island.

Fig. 15 shows dark cells which are comparable to A cells stained with neutral gentian violet. The dark cells are located chiefly at the periphery of the insular cell groups; some of them are arranged in acinus-like structure in the center of which several clear cells are

duct cells have a definite relation to the new formation of the islands. Fig. 20 demonstrates a changed duct.

Bensley recognized the occurrence of an interstitial growth of islands from duct cells in guinea-pigs. We also made similar observations in human pancreas.

A peculiar proliferation of duct epithelium due to unknown conditions was described by Priesel¹⁷; our material presents a certain number of such instances. We found such proliferation in oval or round island-like forms and proved with the aid of serial sections that these cell accumulations were connected with the ducts by a more or less narrow zone of cells. Though they resemble the island structure, they lack capillaries; therefore, they cannot be classed as true islands.

The claim has been made by Weichselbaum and Kyrle,²⁴ Helly⁷ and Bensley,¹ that the duct cells possess the power of forming islands even in adult life. However, until now no definite proof has been offered in the literature to substantiate such statements. We feel that our findings established this fact definitely.

3. ISLANDS SEPARATED FROM THE REST OF THE PANCREATIC TISSUE

The fact that islands exist entirely separated from the rest of the pancreatic tissue and are connected with neighboring structures by blood capillaries only, is the most important factor in support of the theory of internal secretion. However, no definite morphologic proof has been brought forward for the human pancreas to show that the islands are entirely independent structures.

The separate islands we found are limited by fine fibers. Judged by their histologic appearance and micro-chemical proofs, they are normal in every way. Kirkbride⁸ found that islands remaining in the pancreas which becomes fibrosed after ligation of its ducts still show the typical so-called A and B granules. This indicates that, if the islands have some physiologic function, this particular type might show the same activity. Some islands are connected with the rest of the pancreatic tissue by blood capillaries only, which in addition to above considerations leads to the conclusion that they have an internal secretory function in human pancreas. They do not represent, however, an independent organ *sui generis*, because normally not all islands show an independent structure.

favors this view indirectly. Since the acinar cells do change into those of the islands, the reverse process might occur.

Schmidt²¹ and others stated that transition might occur under certain pathologic conditions only. Since we have very often found enlarged islands in normal pancreas and, furthermore, recognized the connection of islands with acini as of common occurrence, we are of the opinion that transition may occur under normal conditions. But though there are some islands connected with acini that do not show distinct morphologic transition, we think both are genetically related, since the infant pancreas contains islands connected with acini more often than the adult pancreas.

2. ISLANDS CONNECTED WITH DUCTS

In the previous paper we maintained that the islands connected with ducts may be classed in two subgroups: (a) The area of continuity between island and duct is very narrow; and (b) the duct and islands are in broad communication.

The former type, shown in our previous paper Figs. 5 and 6, has often been found in the pancreas of infants, especially in new-born individuals, and can be interpreted as persistence of an embryonal structure, in the same way as undifferentiated glomeruli are found in the cortex of the kidney, occasionally even in children of six or seven years. The picture in the congenital syphilitic pancreas supports this explanation. If all acinar cells at the periphery of an island connected with acini become transformed into island cells, such an island may remain connected with the terminal duct. Fig. 19 exemplifies such occurrence most strikingly. Therefore, islands communicating with ducts over narrow strips will also be interpreted as resulting from such transitions of acinar cells.

However, the islands connected with ducts at a wide circumference differ from the former type. They have been found in adult pancreases and never in those of infants. This leads us to believe that they are not embryonal remnants (see Fig. 8 in the first paper¹⁶). One-half of the epithelial lining of a duct does not show true duct-cell character; the cells are rather similar to those of the islands; such cell groups have no basement membrane dividing them from the island cells. Islands of this type are usually small and show an undifferentiated structure. We assume from such findings that the

activity of the islands. They cannot be accepted, however, as constituting an organ *sui generis*, since normally most of the islands are connected with acini which are capable of changing into island cells.

6. The new formation of the islands in adult pancreas may occur by transformation and hyperplasia of the acinar, centroacinar or duct cells.

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4. NEW FORMATION OF THE ISLANDS IN THE ADULT PANCREAS

That the acinar cells are able to change into island cells is an undeniable fact. The homogeneous cells, intermediate in type between the acinar, centroacinar and island cells, not only occur in groups but a hyperplasia of these cells does exist. This supports the conception that a new formation of islands may occur by transformation of acinar and centroacinar cells in adult life.

We also assumed that the duct cells are capable of forming islands. Since the centroacinar cells belong to the duct in a broad sense,¹⁸ there are really two elements which play a part in the formation of the islands, namely, acinar and duct cells. It does not seem plausible to have the islands derived from two different elements, *i. e.*, to accept a dualistic view with regard to their formation. However, if one examines the undifferentiated pancreas of the new-born, such can easily be conceived (see Figs. 4 and 7 in the previous paper¹⁶), because such islands are connected with acini as well as ducts; furthermore, the argentophilic granules can be found in the acinus as well as in the duct cells.

CONCLUSIONS

1. The process of hypertrophy of the islands of Langerhans in the adult pancreas is mostly due to transformation and hyperplasia of acinar and centroacinar cells, the hyperplasia of the island cells proper being relatively insignificant.

2. The acinar cells are able to change into island cells. The reverse process might occur, though it is difficult to prove morphologically.

3. In this respect, the direct contact between islands and acini indicates a genetic relation.

4. One type of island connected with ducts is interpreted as a remnant in the postembryonal pancreas or as resulting from the transformation from acinus to island cells, while the other type has a definite relation to the new formation of islands in the adult pancreas.

5. The fact that islands entirely separated from the rest of the pancreatic tissue do exist under normal and pathologic conditions may be regarded as morphologic evidence of an internal secretory

FIG. 8. Case 73, 71 years. Hemorrhage of middle cerebral artery. Exceedingly irregularly-shaped island cell groups. The light cell groups in lower part indicate original island, in the upper part newly forming island. Dark cells are acinus cells. $\times 400$.

PLATE 52

FIG. 9. Case 73. Homogeneous cells in group. $\times 550$.

FIG. 10. Case 66, 30 years. Stenosis of mitral valve. Homogeneous cell group, showing a mitotic figure of a centroacinar cell. $\times 800$.

FIG. 11. Case 17, 52 years. Pneumonia. Homogeneous cell group, within which few dark cells (basophilic) still remain. The dark cells are situated around the homogeneous cell group (compare with Fig. 15). $\times 550$.

FIG. 12. Case 73. The picture was taken from the periphery of a very large island, which presents homogeneous cells in groups. $\times 500$.

PLATE 53

FIG. 13. Case 73. The homogeneous cell groups, showing various stages of transformation from acinar cells. $\times 600$.

FIG. 14. Case 73. An island-like cell group, containing few acinar cells. $\times 500$.

FIG. 15. Case 8, 34 years. Peritonitis after perforated duodenal ulcer. An apparently newly forming island, showing dark cells, which are comparable to so-called A cells, located mostly around the light cells. Acinus-like structure is seen at the lower portion. Fixed in 20 per cent formalin, stained with Kultschitzky's hematoxylin after treatment with potassium bichromate. $\times 400$.

FIG. 16. Case 56, 8 years. Uremia. Island cells contain argentophilic granules (lower portion), the acinar cells remain clear. An isolated argentophilic cell within the acinus (upper part). Section was kept in 2 per cent silver solution five days for impregnation and then stained with hematoxylin. $\times 800$.

PLATE 54

FIG. 17. Case 56. A pancreatic duct cell showing argentophilic granules (in the center). $\times 800$.

FIG. 18. Case 82, 27 years. Postoperative shock (tenorrhaphy of left hallux). This pancreas contains small islands connected with acini throughout. Both islands are cut at their widest diameter as seen on serial sections. $\times 120$.

FIG. 19. Case 56. An island connected with the terminal duct. $\times 540$.

FIG. 20. Case 77, 50 years. Carcinoma of bladder. An island-like structure, showing connection with duct. $\times 380$.

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DESCRIPTION OF PLATES

Preparations illustrated in Figs. 1, 2, 3, 5, 16, 17, 18 and 19 were made by the silver impregnation method, followed by hematoxylin-eosin. All others except Fig. 15 were stained with hematoxylin-eosin.

PLATE 50

- FIG. 1. Case 16, 27 years. Insufficiency of mitral valve. A very large island. In the lower portion there is a peculiar structure like the arm of an octopus. This is part of an adjoining island. $\times 140$.
- FIG. 2. Case 16. This picture was taken from the periphery of a very large island, showing fibers not containing capillaries. $\times 500$.
- FIG. 3. Case 17, 52 years. Pneumonia. This island shows gradual transition into acini, especially the upper portion. $\times 500$.
- FIG. 4. Case 75, 35 years. Endocarditis. Cell changes at the connection of island and acini.

PLATE 51

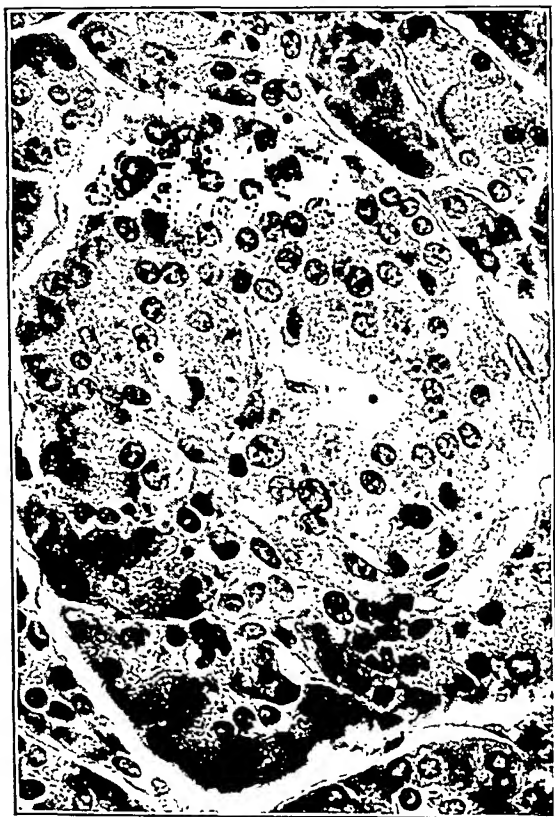
- FIG. 5. Case 72, 3 years. Pneumonia. Section was cut from the head of this pancreas. A dumb-bell-like fusion of two islands. $\times 140$.
- FIG. 6. Case 49, 63 years. Carcinoma of the common bile duct. The picture demonstrates the periphery of a very large island, showing glandular structure of island cells (in the center), dark cells within the acinus and dark cells within the island. $\times 500$.
- FIG. 7. Case 17. Photomicrograph was taken from the periphery of a very large island, showing complicated cell changes; dark cells (in the center), capillary in the basement membrane of acini (upper part) and a cell group with lumen suggesting a terminal duct (lower portion slightly to the right). $\times 600$.



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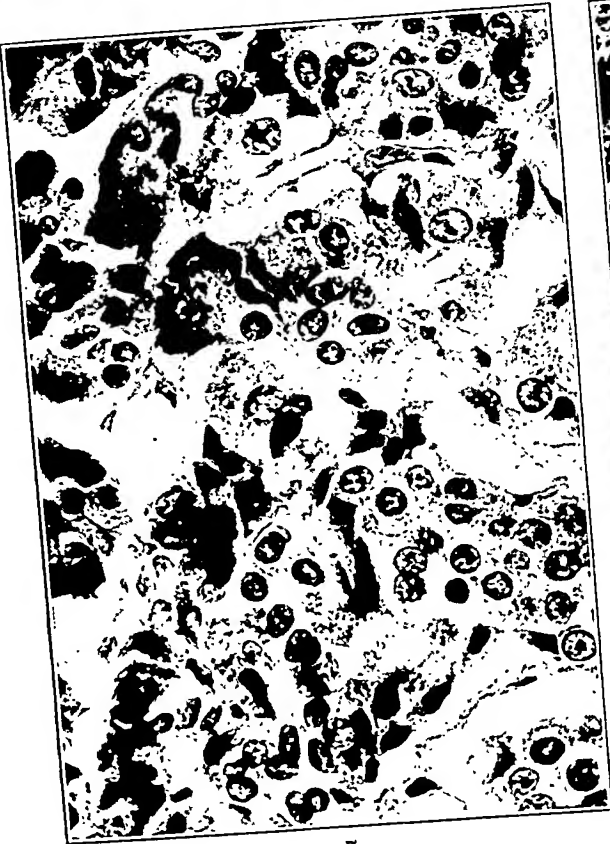
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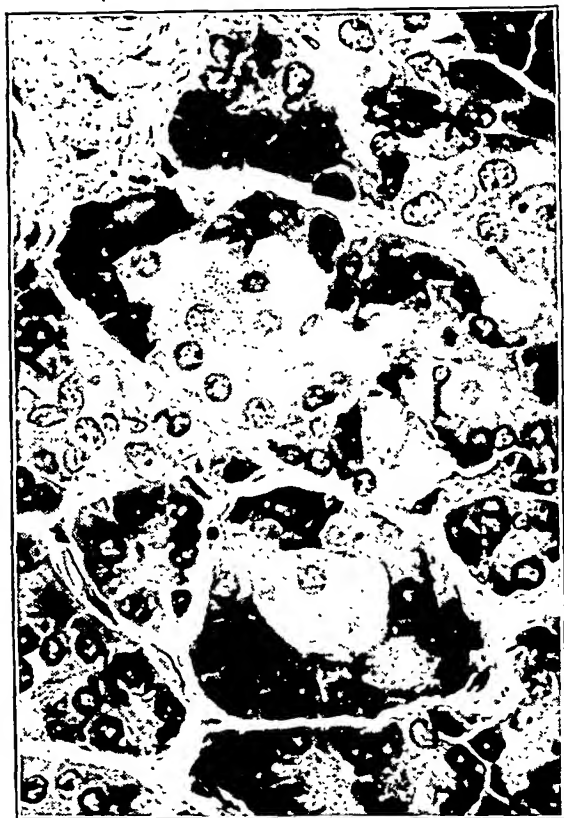


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Islands of Langerhans in Human Pancreas



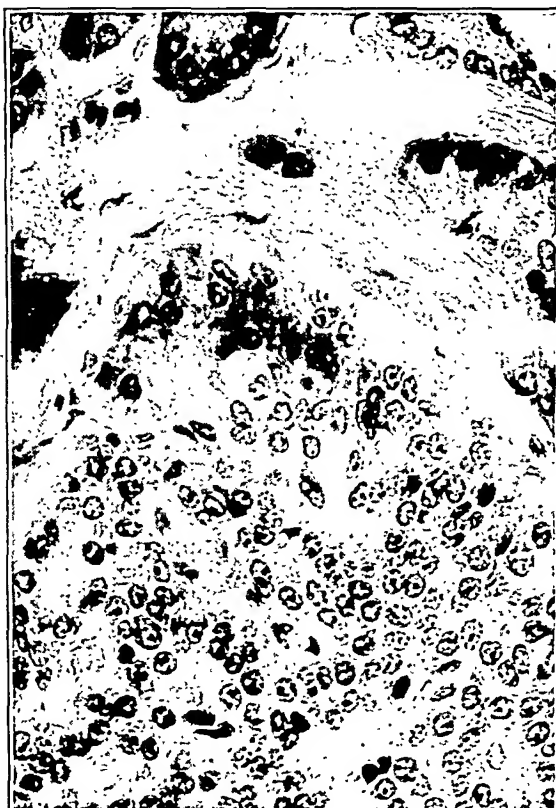
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Otani

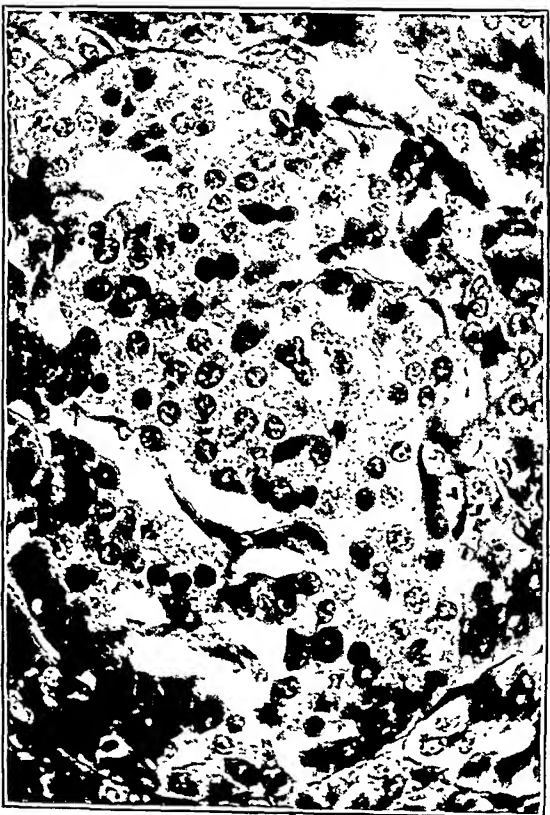
Islands of Langerhans in Human Pancreas



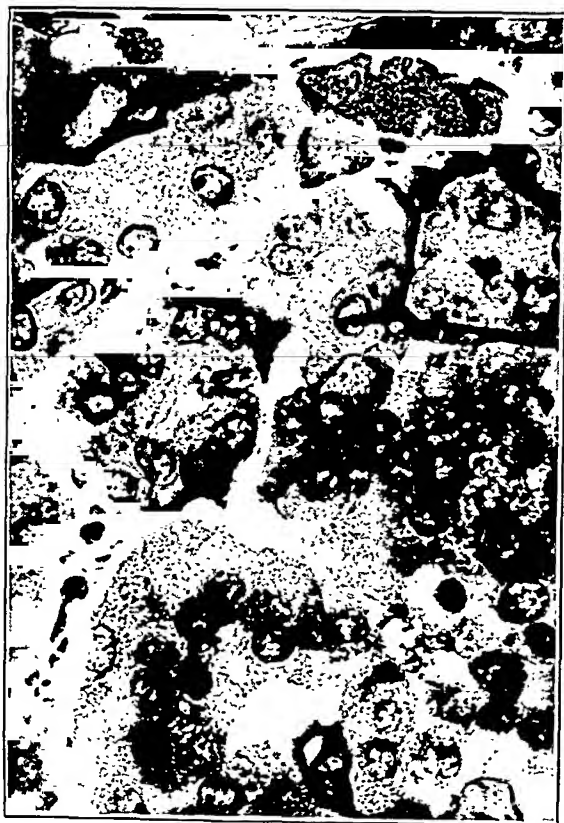
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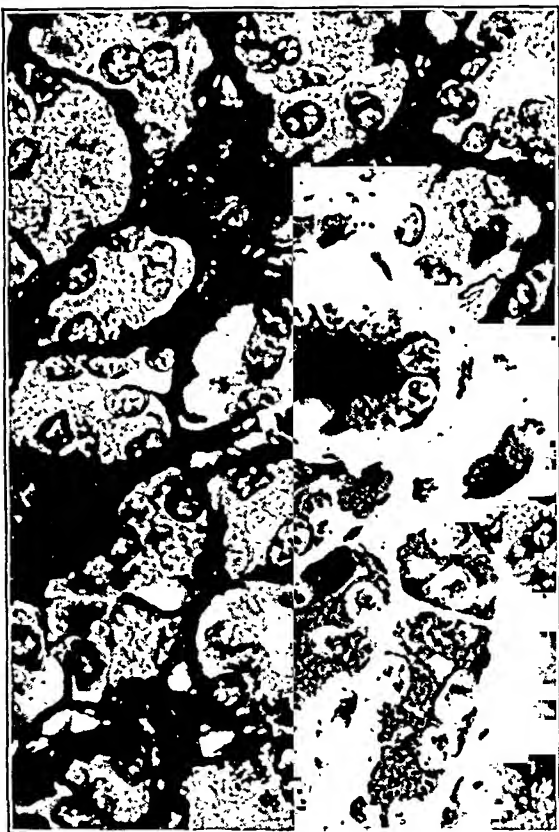
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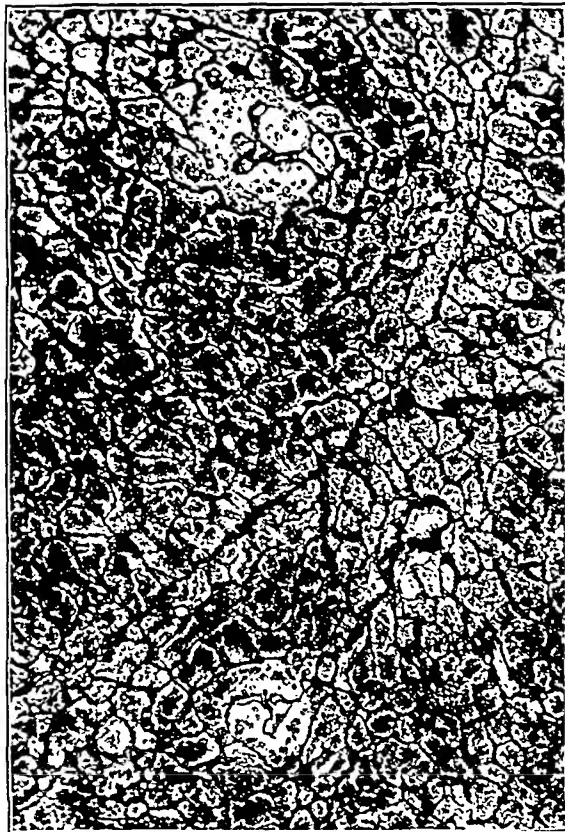
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Islands of Langerhans in Human Pancreas



17



18



19

Otani



20

Islands of Langerhans in Human Pancreas

frontal headache, constipation, vomiting and anorexia. No abdominal pain. No chills or perspiration. Extreme thirst.

Physical Examination. Patient looks acutely ill. Skin has a distinct brownish tinge. There is marked cyanosis. Mouth dry. Tonsils large and succulent. Chest examination negative. Heart normal. Pulse rapid and regular. Blood pressure: systolic, 80 and diastolic, 48. Abdomen soft. Spleen and liver palpable. Skin dry and rough. Reflexes normal. Urine: albumen, trace. Blood: hemoglobin, 86 per cent; red blood cells, 5,330,000; white blood cells, 14,400; polymorphonuclears, 77 per cent; lymphocytes, 20 per cent; mononuclears, 3 per cent. Widal negative.

April 30. Patient apparently somewhat improved. No further positive findings. Blood pressure: systolic, 70 and diastolic, 36. Only positive findings are circulatory depression, pigmentation of the skin and dehydration. Urine: albumen, trace. Blood: white blood cells, 6,200; polymorphonuclears, 57 per cent; lymphocytes, 29 per cent; and mononuclears, 10 per cent.

May 2. Patient shows no improvement. Spinal puncture negative. Blood pressure: systolic, 73 and diastolic, 36. Urine: albumen, trace. Blood: hemoglobin, 74 per cent; red blood cells, 4,450,000; white blood cells, 16,900; polymorphonuclears, 65 per cent; and lymphocytes, 23 per cent. Blood culture negative. Vomitus contained some blood. Stool positive for occult blood.

May 3. Patient died.

Clinical Diagnosis. Addison's disease.

NECROPSY. Thirty-six hours after death. The essential findings of the necropsy are as follows.

Thymus. Weight 49 gm. Hyperplastic.

Lymphoid Tissue. Intestinal lymph follicles markedly enlarged. Mesenteric lymph nodes the size of almonds. The tonsils are large. Microscopic sections show hyperplastic lymphoid tissue.

Skin. Appears pigmented and dry.

Adrenals. The organs are small and firm. Microscopic examination shows marked atrophy of the cortex with large areas in which there is no adrenal parenchyma. There is marked fibrosis of the cortex and medulla. Extensive lymphocytic infiltration exists throughout the organ. Both organs present a similar appearance. No tuberculosis is found. Semilunar ganglia normal.

Spleen. Weight 270 gm. Measures 12.5×8×3 cm. The organ is

A REPORT OF TWO CASES OF ESSENTIAL ADRENAL INSUFFICIENCY (ADDISON'S DISEASE)*

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Addison's disease is a clinical entity in that it represents a deficiency of adrenal function to a level incompatible with life. Associated with this adrenal insufficiency there is usually manifested a pigmentation of the skin and mucous membranes, languor and lassitude, hypotension and quite often a derangement of gastro-intestinal function. Extensive destruction of adrenal tissue is always present in Addison's disease but it is quite apparent that this destruction is not always caused by the same agent. From the pathology of the adrenal the cases have been divided (Bittorf) into two essentially different groups. The first group is that of essential or idiopathic adrenal insufficiency in which no infectious agent or tumor growth plays a part. The second group is that of secondary adrenal insufficiency and comprises all cases in which infections, such as tuberculosis, or tumor growths so derange the adrenal function as to cause, clinically, the symptom-complex originally described by Addison.

In a survey of the medical literature of the United States, all the cases of Addison's disease found recorded with necropsy findings fall into this second group. Warthin¹ in a paper on thymic hyperplasia briefly mentions three cases which belong to the first group. During the past year in the service of the Wisconsin General Hospital there have occurred two cases of essential or idiopathic adrenal insufficiency. The following is a brief clinical and necropsy record of these two cases.

HISTORIES OF CASES

CASE H. Male, age 28, single, Canadian. Graduate student in the College of Agriculture. Admitted to student infirmary April 27, 1926. Patient ill one week. Chief complaints were weakness, severe

* Received for publication November 10, 1926.

Lungs. Both lungs show puckered scars at the apex (healed tuberculosis) and small areas of bronchopneumonia.

Spleen. Weight 125 gm. Large in comparison with the size and weight of the other organs. Malpighian corpuscles large and hyperplastic on microscopic examination.

Liver. Weight 1040 gm. Appears normal.

Pancreas. Appears normal in gross. Section shows the islands of Langerhans more numerous and larger than usually seen.

Gastro-Intestinal Tract. Lymphoid follicles and Peyer's patches large.

Kidneys. Weight 153 gm. Normal.

Adrenals. No adrenal glands could be demonstrated on gross examination and no accessory adrenal tissue could be found around or in the kidneys, along the ureters or in any of the pelvic tissue. The tissue in the region where the adrenals are normally found was dissected out and preserved for microscopic study. Section of this tissue showed the structure of the adrenal gland with here and there small clumps of cortical or of medullary adrenal tissue. Throughout the glands there was extensive lymphocytic infiltration. The areas which contained adrenal parenchyma showed some of the parenchymal cells necrotic and invaded by mononuclear and polymorphonuclear leucocytes. No evidence of tuberculosis was found. Semilunar ganglia negative.

Ovary. Small simple cysts.

Diagnoses. Marked atrophy and destruction of adrenal tissue associated with hyperplastic lymphoid tissue; old rheumatic endocarditis; old healed bilateral pulmonary tuberculosis; bronchopneumonia; and hyperplastic islands of Langerhans.

DISCUSSION

Essential or idiopathic adrenal insufficiency with the Addisonian syndrome appears to occur more commonly in European countries than in America, if one may judge from the literature.

Wiesel² after a study of several cases of Addison's disease came to the conclusion that the primary lesion was in the chromaffin tissues. In most of his cases he noted the presence of hyperplastic lymphoid tissue with thymic and splenic enlargement.

Hedinger³ in an analysis of fifteen cases of Addison's disease, fourteen of which showed tuberculosis of the adrenals, found a com-

firm and dark red. The capsule is not thickened. On section the organ appears congested and the Malpighian corpuscles are very prominent. Microscopic examination shows congestion and hyperplastic lymphoid tissue.

Testes. Normal.

Diagnoses. Status thymico-lymphaticus; atrophy and fibrosis of adrenals.

CASE B. Female, age 29, housewife. Has always lived in Wisconsin. Entered hospital April 21, 1926. Chief complaint was weakness. There has been progressive pigmentation of the skin since 1924. Pigmentation of the gums appeared at the same time. There have been numerous colds with chills and fever. Backache has been marked. There has been no pain in the chest and no night sweats, cough or hemoptysis. One month ago the patient had an attack of profuse sweating associated with delirium for one day. The sweating was largely localized to the face. Since this attack the patient has been nauseated and has had several attacks of vomiting. During the past four years the patient has lost 40 pounds. There are three children, aged 4 years, 2 years, and 11 months, living and well. There were two miscarriages.

Aside from marked pigmentation of the skin and the mucous membrane, *physical examination* revealed nothing of significance except a low blood pressure. Blood pressure: systolic, 90 and diastolic, 50; systolic, 92 and diastolic, 52; systolic, 80 and diastolic, 50. Blood sugar, 72, non-protein nitrogen, 27.3 and uric acid 41.3. Wassermann negative. Basal metabolism: -10 to -16. Temperature, pulse, respiration and urine normal. Blood count: red blood cells, 4,280,000; white blood cells, 5,500; polymorphonuclears, 39 per cent; eosinophiles, 3.6 per cent; lymphocytes, 53 per cent; and mononuclears, 4 per cent.

During stay in the hospital there were several attacks similar to the one a month before admission. The patient failed to respond to ephedrine, suprarenal gland and stimulants and died April 25, 1926.

Clinical Diagnosis. Addison's disease.

NECROPSY. Two hours postmortem. Limited to abdominal incision.

Heart. Weight 156 gm. There is an old mitral rheumatic endocarditis.

animal after suprarenalectomy." He also states: "The mechanism involved in the regeneration of the thymus which follows suprarenalectomy is still not understood, and while thymic hyperplasia may be one manifestation of the generalized lymphoid hyperplasia that follows sublethal but sufficient suprarenal injury, nevertheless, the fact should not be lost sight of that thymic enlargement may represent a specific reaction of this organ to suprarenal injury."

From the above brief survey of the literature the two cases here recorded show several points of interest. They both belong to the "essential or idiopathic adrenal insufficiency," as classified by Bitorf. In both cases there was present a marked hyperplasia of the lymphoid apparatus, including the spleen in both cases and the thymus in the one case where examination was possible. Neither case showed any clinical or pathologic evidence of hypofunction of the gonads. In the case of the female, pregnancy and healthy children had ensued. In the case of the male, the testicles were functional and appeared normal in the gross and the microscopic examinations.

These cases emphasize the common occurrence of the pathologic picture of status thymico-lymphaticus in association with Addison's disease. That cases of status thymico-lymphaticus occur without the clinical syndrome of Addison's disease is common knowledge. The experimental evidence cited above gives proof of thymic regeneration and general lymphoid hyperplasia where sublethal suprarenal injury has been produced. It seems quite plausible, therefore, that the Addisonian syndrome is produced only in cases of extreme adrenal insufficiency and that status thymico-lymphaticus is but an indication of adrenal insufficiency, being present in both sublethal and lethal cases.

The etiologic factor or factors causing adrenal insufficiency, aside from destruction of the adrenal tissue by infections, such as tuberculosis, or by tumor growths within the organ, are not at present understood. A popular conception is that there is a disturbed balance between the secretions produced by the ductless glands. Whether this is the real factor remains to be proved. If, however, status lymphaticus may be taken as an indication of hypofunction of the adrenals, the greater frequency of the lymphatic constitution in children and young adults would suggest these cases have deficient adrenals at birth. The degree of deficiency would, in association

mon occurrence of the picture of status thymico-lymphaticus. He agrees with Wiesel that the essential pathologic lesion in these cases is in the chromaffin tissue.

Bittorf⁴ collected from the literature forty-seven cases of Addison's disease in which necropsy had shown absence of tuberculosis or tumor growth in the adrenals. To this series he added five cases of his own, three of which came to necropsy and showed the same condition. In a study of the adrenal in these cases, three showed involvement of the cortex only, and the remainder showed involvement of the whole organ. In all but one case there was present a hyperplastic condition of the lymphoid tissues. Both sexes were about equally involved, there being twenty-three males and nineteen females. The age range was 10 to 50 years in the male and 18 to 65 years in the female. In the majority of instances in which the sympathetic nerve ganglia were studied, these structures appeared normal. Bittorf's conclusion is that from the anatomic side the essential lesion is a disease of both adrenals and that the sympathetic nerve system is seldom involved. He also believes there is a relation between status thymico-lymphaticus and Addison's disease.

Marine, *et al.*,⁵ from evidence obtained by removal of the adrenals, gonads and thyroid in the rabbit, conclude that removal of the thyroid hastens the involution of the thymus and removal of the adrenals or gonads not only retards this involution but brings about a condition where there is an actual regeneration of the thymus. Removal of both the gonads and adrenals produces this result more strikingly than the removal of the one or the other. They conclude that "the so-called lymphatic constitution which underlies or accompanies exophthalmic goiter, Addison's disease, and acromegaly also appears to be dependent on a partial suppression of certain functions of the inter-renal and sex glands."

Jaffe⁶ obtained similar results as the above in rats following removal of the adrenals. He states: "It is now generally accepted that both in Addison's and in Grave's disease regeneration of the involuted thymus occurs, which may take place even in the presence of profound emaciation or chronic infection. We are of the belief that the large thymus which occurs in status lymphaticus, and the regeneration which occurs in Addison's and Grave's disease are brought about by the same disturbances of glandular inter-relations which bring about regeneration of the thymus in the experimental

CONCLUSIONS

Two cases of essential adrenal insufficiency with the Addisonian syndrome are recorded.

The association of status lymphaticus and hypofunction of the adrenals is further emphasized.

An impression is gained that status lymphaticus is an expression of an attempt on the part of the body to correct a deranged metabolism brought about by a paucity of adrenal secretion.

The cases here recorded gave no clinical or pathologic evidence of gonad insufficiency.

I wish to express my appreciation to the medical service of the Wisconsin General Hospital and of the Student Infirmary for the privilege of incorporating in this article a brief summary of the clinical history of the cases herein reported.

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DESCRIPTION OF PLATE

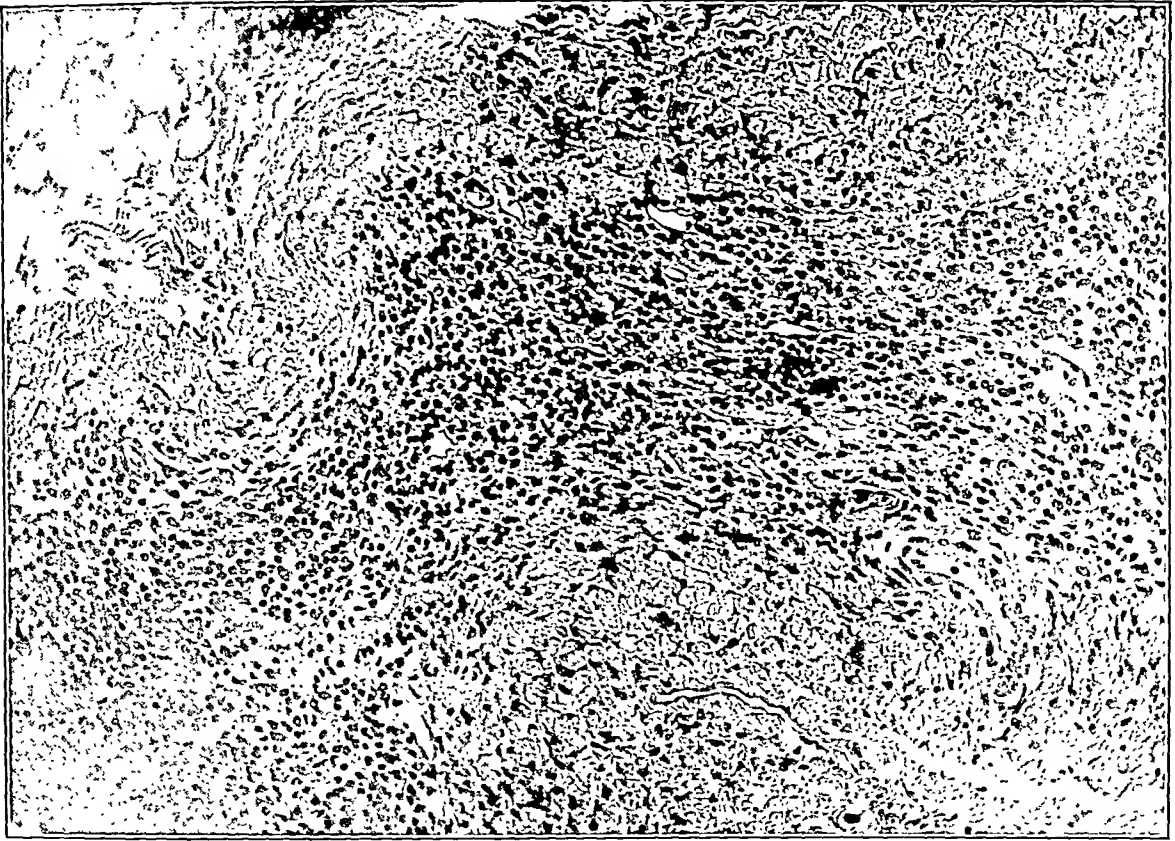
PLATE 55

- FIG. 1. Adrenal from Case B, showing absence of adrenal tissue and marked lymphocytic infiltration of the organ. $\times 100$.
- FIG. 2. Adrenal from Case H, showing a few parenchymal cells of the cortex. Some of these cells are markedly hypertrophied. There is also considerable lymphocytic infiltration of the organ. $\times 250$.
- FIG. 3. Adrenal from Case B, showing a few of the parenchymal cells of the medulla still present. Note the extreme degree of lymphocytic infiltration. $\times 300$.

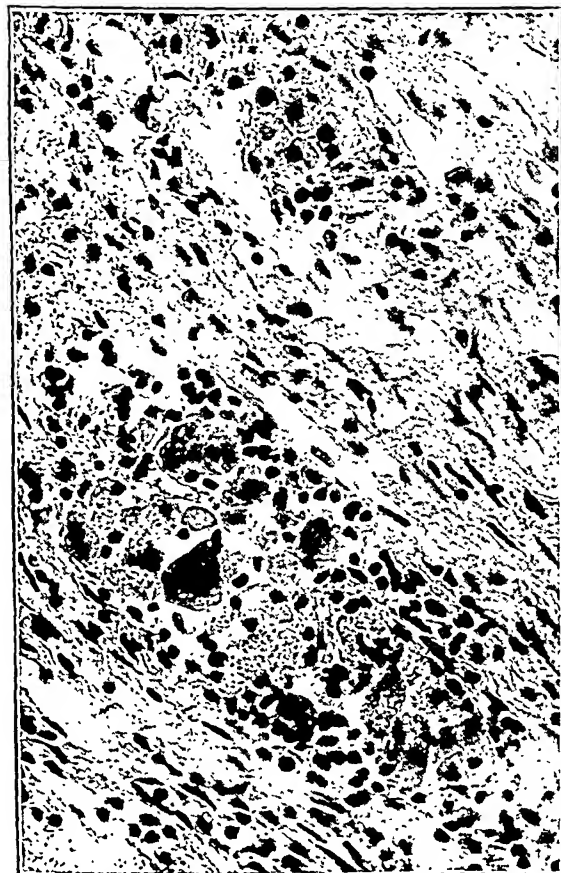
with the mode of life, determine the date at which clinical manifestations of adrenal insufficiency would appear. Gross and microscopic examination of the adrenals in many of these cases would be negative.

The fact that toxic necrosis of the adrenal parenchyma occurs in various infectious diseases has been clearly demonstrated by Graham⁷ and others. In such cases there is evidence of regeneration of the parenchymal cells. It seems apparent that, because of the absence of clinical manifestations of adrenal insufficiency subsequent to recovery from these infections, the regeneration following necrosis is sufficient to permit of normal adrenal function. Although in the two cases here recorded there did not appear to be any etiologic connection with any infection, still it must be considered a possibility that during some infection there had occurred extensive destruction of the parenchyma of the adrenal with insufficient regeneration for continued proper adrenal function.

The hyperplastic condition of the lymphoid tissues may be explained in one of two ways, the first being that the adrenal under normal conditions exerts a suppressive or regulating effect upon the thymus and other lymphoid tissues. This view is expressed by Jaffe and Marine, *et al.* The second explanation is that, with the removal of the adrenal secretions, toxic products of metabolism accumulate and that cells of the lymphoid tissues are called out in large numbers in an attempt on the part of the body to detoxify such substances. The rôle that the lymphocytes play in various reparative processes in the body would make it seem that this second explanation of the lymphoid hyperplasia were the more plausible. Also the extensive lymphocytic infiltration of the damaged adrenals would suggest a reparative process. There appears to be no evidence of lawlessness of growth in the hyperplastic lymphoid tissue in these cases. There appears, however, to be a far greater demand for the lymphoid elements in adrenal insufficiency than in other disease processes. This explanation would also fall in accord with Warthin and others who have noted a lymphoid exhaustion in a large percentage of these cases.



1



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Medlar



3

Essential Adrenal Insufficiency

and B strains the rats used belonged to the 37th and 38th inbred generation (subseries I), or to the 46th and 47th generation (subseries II). In further experiments, instead of using not directly related rats we carried out the same transplantations between brothers in the A as well as in the B strain, and again earlier (40th and 41st generation) as well as later generations (46th and 47th generations) were used. Lastly as control experiments we transplanted tissues from A to B strain and from B strain to A strain, in the earlier as well as in the later generations.

We see thus that the inbreeding within the rat strains A and B has progressed much further than within the guinea-pig strains. In the case of the guinea-pigs we used, in the large majority of our experiments, animals belonging to generations varying between the 15th and 23rd, while the rats used were from generations ranging between the 37th and 47th. We should therefore have expected that the similarity between the individuality differentials of donor and host would be much closer in the latter than in the former; however, our experiments proved that the opposite condition holds true.

SERIES A. TRANSPLANTATIONS FROM RATS OF STRAIN A TO OTHER RATS OF STRAIN A

In these experiments the donor and host did not belong to the same litter; they were therefore not brothers or sisters, nor did the exchange of tissues take place between parents and children; however a more distant relationship existed in all cases. Donor and host belonged to different litters which represented the 37th or the 38th generations, although in some cases the litter of the donor belonged to one, and the litter of the host belonged to the other, of these generations.

Subseries I, in which rats of the 37th and 38th inbred generation were used. Twenty experiments were made in this series; time of removal of transplant from host and examination of transplant varied in different cases between 19 and 45 days, the large majority having been examined between the 24th and 31st day. In eight cases a homoio-reaction was obtained; in two further cases the reaction was only slightly less intense. In six cases a syngenesio-reaction was found and in four cases the result approached conditions characteristic of autotransplantation. In 50 per cent of the cases

I. TRANSPLANTATION AND INDIVIDUALITY DIFFERENTIAL IN STRAINS OF INBRED RATS*

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In a preceding paper one of us has reported on the transplantation and individuality differential in inbred families of guinea-pigs which were obtained from the Department of Agriculture in Washington.¹ In this paper we wish to report on similar experiments in rats and while it is published subsequently to the corresponding paper on guinea-pigs, the experiments on inbred rats were begun at an earlier time than those on guinea-pigs. However, the results in the latter were so much more in accordance with expectations, that it was thought best to publish them first in order to contrast with them the results obtained in rats. The rats used had been inbred by Miss King in the Wistar Institute, Philadelphia,² and the experiments on both guinea-pigs and rats were carried out in the Department of Pathology and Comparative Pathology of Washington University Medical School.

Two strains of rats, originally derived from the same lot, were inbred through many successive brother and sister matings. Thus a strain "A" and a strain "B" of inbred rats were separated. However, there existed a difference in the mode of inbreeding in rats and guinea-pigs; in the case of the rats there was selective breeding, in each case the most vigorous individuals being used, while in the case of the guinea-pigs the breeding was not selective. Thus Miss King did not observe a deterioration in the vigor of the rats in the course of long continued inbreeding, while in the case of the guinea-pigs a definite, although on the whole moderate, deterioration has been established by Dr. Wright.

We carried out the following series of experiments. Series A: We transplanted tissues from rats of inbred strain A to other not directly related A rats. Series B: In a similar way we carried out corresponding transplantations within the B strain. In both the A

An accident has made it necessary to publish this paper first. The report on the transplantation and individuality differential in inbred guinea-pigs will soon follow.

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into center of piece. The central parts of thyroid ring are intensely infiltrated by lymphocytes, so that the appearance of a lymph gland is produced. Also in the peripheral parts of the thyroid ring there are some collections of lymphocytes. Grade 3.5.

6. Thyroid transplant well preserved; acini with or without colloid. Fat and a small amount of connective tissue in center. Well preserved parathyroid; in the center of it are some masses of lymphocytes around vessels. Lymphocytic infiltration in one pole has destroyed a considerable amount of thyroid tissue; but on the whole the lymphocytic infiltration is not very pronounced. Ovary shows good germ epithelium cyst. Well preserved large follicles; also atretic large follicles and follicles in the stage of connective tissue abresia; some primordial follicles with preserved ova. Yellow interstitial tissue. Medullary ducts with preserved Fallopian tubes well preserved; occasionally a few polymorphonuclear leucocytes in lumen. In the connective tissue in and around ovary slight lymphocytic infiltration. In uterus unstriated muscle tissue preserved. Grade 4.50.

7. In another similar transplant there are in addition to above findings new corpora lutea with many capillaries formed in the transplanted ovary. Grade 4.75.

8. *45 days*. Uterus transplant shows glands markedly proliferating with high epithelium. Some dilated glands with flat epithelium. Certain gland cells secrete mucus. Fibrillar connective tissue around glands. A number of lymphocytes migrate through epithelium. In places a few polymorphonuclear leucocytes in the interstitial tissue. Connective tissue cells and some lymphocytes in mucosa around epithelium, the latter lymphocytes migrating into the epithelium. A great amount of unstriated muscle preserved. In center of transplant hyaline fibrous tissue. Fallopian tubes almost unchanged; epithelium forms papillae. In ovary a great quantity of so-called interstitial gland consisting of large fairly vacuolar cells, with yellow pigment. A few primordial follicles with eggs. Some degenerating eggs in other follicles. Large system of medullary canals and germ epithelium prominent. Some nests of cells without ova in cortex. Fat tissue with blood pigment. Around a vessel in ovary a collection of lymphocytes. Grade 5.25.

9. *31 days*. Thyroid. Acini preserved with colloid; also parathyroid preserved. Large blood vessels surrounded by lymph

we obtained therefore a marked reaction against the transplant on the part of the host.

The grades in the various experiments were as follows: 3.25; 2; 1; 2.5; 1.5; 5.75; 3.5; 3; 5.5; 5; 3.25; 3.5; 4.50; 1; 1; 4.75; 5.25; 4.25; 3.5; 3.75. The average grade was 3.4. We shall cite a number of abstracts of the results obtained.

1. *24 days.* Thyroid transplant shows large masses of acini, either with or without colloid. There is dense fibrous tissue in the center. Between the ring of acini and center and in periphery of transplant there is a very marked lymphocytic infiltration; lymph vessels are filled with lymphocytes. In the cartilage transplant there is much lymphocytic infiltration in the fat tissue and in the connective tissue around the cartilage. Some lymphocytes penetrate into perichondrium and into newly formed cartilage, destroying the margin of it. Where cartilage is necrotic, perichondrium produces new cartilage around it. Some connective tissue penetrates between necrotic and regenerating cartilage. Grade 3.25.

2. *24 days.* The central part of thyroid is composed of hyaline connective tissue; the periphery of transplant consists of masses of lymphocytes among which are a few compressed acini; almost all the acini have been destroyed and their place taken by lymphocytes. In cartilage transplant, the cartilage is on the whole well preserved; where parts are necrotic, regeneration and a new formation of cartilage has taken place. In the surrounding fat tissue and connective tissue there is much lymphocytic infiltration. In some places lymphocytes penetrate to perichondrium. Grade 2.

3. *22 days.* Thyroid transplant destroyed; only fibrous tissue with lymphocytes left. In cartilage transplant, definite lymphocytic infiltration in surrounding connective tissue. Perichondrium produces new cartilage. Grade 1.

4. *25 days.* In thyroid transplant a good ring of acini, closely adjoining each other; good colloid. Loose connective tissue and large vessels in center. No lymphocytes. Cartilage surrounded by fat tissue. Around necrotic areas some perichondrial regeneration of cartilage. In the fat tissue some collections of lymphocytes. Grade 5.75.

5. *29 days.* Many acini of thyroid transplant with good colloid; some are without colloid. A number of acini in ring still closely adjoining each other; well developed vessels penetrate through ring

COMMENT. The average grade of the earlier experiments with the 37th and 38th generation is 3.4; that of the later experiments with the 46th and 47th generation is 1.12. Of course the number of experiments in the latter subseries is only four. The average grade of results in Series A (both subseries combined) is 3. Transplants of thyroid, parathyroid, cartilage, uterus, Fallopian tubes and ovaries were used as a test in grading the severity of the reaction on the part of the host. The behavior of these organs does not differ in any essential respect from other previous findings which one of us described in earlier papers. Lymphocytes and connective tissue of the host invade and damage the tissues; in addition a direct injurious effect of the homoio-toxins may be observed in the case of sensitive tissues. The ovary is usually invaded by lymphocytes to a much less extent than thyroid or parathyroid. Corpora lutea may develop through rupture of the follicles; in addition atresia may take place in follicles which do not have a chance to rupture. In general there is again a parallelism in the behavior of all the tissues from one animal which are transplanted into another. We shall compare the results obtained in this series with those obtained in the case of non-inbred rats, after we have reported on all the experiments with inbred rats.

SERIES B. TRANSPLANTATIONS FROM RATS OF STRAIN B TO OTHER RATS OF STRAIN B, BELONGING TO DIFFERENT LITTER

Subseries I, in which rats of the 37th and 38th generation were used. There were twenty-two experiments. The pieces were removed at periods varying between 14 and 37 days following transplantation. In two cases the results approached an auto-reaction; in seven cases a syngenesio-reaction was obtained; in the remaining thirteen cases the result corresponded to a homoio-reaction or approached this condition. The grades in the individual experiments were as follows: 2.75; 1.25; 1; probably 1-2; 4; 1; 2; 4.25; 4; 2; 5; 5; 1.5; 1; 1; 5; 1.25; 5.50; 5; 4.5; 5.50. Average grade 3.0 (or 3.1). We shall cite some examples which illustrate especially the behavior of organs other than thyroid and cartilage.

1. 36 days. Inbred rats of the 36th generation. Thyroid not preserved; only fibrous tissue with lymphocytes. Ovary contains medullary ducts which are much infiltrated with lymphocytes; also

vessels which are filled with lymphocytes. A great deal of lymphocytic infiltration, in places very dense. Lymphocytes seem to migrate from the tissue into the blood vessels rather than in the opposite direction. In transplanted uterus and Fallopian tube muscle tissue preserved. Epithelium surrounded by dense fibrous tissue; both structures noticeably infiltrated with lymphocytes. In the walls of the tubes also some lymphocytic infiltration. In the surrounding transplanted fat tissue especially large masses of lymphocytes. One ovary well preserved. Germ epithelium, tunica albuginea and yellow interstitial tissue preserved; also medullary ducts. In tunica albuginea small apparent follicles consisting of granulosa cells without ova. Degenerated corpus luteum with very vacuolar and a few preserved lutein cells. On the whole, little lymphocytic infiltration in this ovary. The other ovary is well preserved also. Small follicles with ova; large follicles with large cavities and good ova. Germ layer partly infiltrated with lymphocytes; many mitoses in germ epithelium. In general slight, but in places more marked, lymphocytic infiltration in this ovary. Grade 4.25.

Subseries II. Inbred rats of the 46th and 47th generation were used in four experiments. The pieces were in all cases removed for examination thirty days after transplantation. Grades 1; 1; 1.25; 1.25. The results were those characteristic of typical homoio transplantation.

We shall cite two abstracts. 1. *30 days.* From rat of third litter of 46th generation to first litter of 47th generation. In thyroid transplant only fibrous tissue found. Around cartilage increase of fibrous tissue and a discontinuous, but distinct, mantle of lymphocytes. Connective tissue and lymphocytes invade cartilage; instead of areolar and fat tissue we find fibrous tissue. Muscle is necrotic. Bone marrow is replaced by connective tissue with lymphocytes. Around bone, giant cells and lymphocytic infiltration. Vessels, connective tissue and lymphocytes penetrate into bone. Grade 1. 2. *30 days.* From first litter of 47th generation to second litter of 46th generation. Thyroid transplant shows only fibrous tissue. Around necrotic cartilage large plate of perichondrial cartilage. Much fibrous tissue and many coalesced fat cells. In areolar tissue much newly formed connective tissue with collections of lymphocytes. Around cartilage, moderate and discontinuous, but distinct, mantle of lymphocytes.

part of the transplanted tissue is necrotic. In the peripheral connective tissue are collections of bile ducts which are surrounded by fibrous or dense fibrillar connective tissue. There are areas of liver cells around bile ducts and around vessels. Some of these cells have two nuclei and some become vacuolar. Lymphocytes are found lying around liver cells and some may penetrate into the latter. In peripheral fibrous tissue are lymph vessels with lymphocytes and a certain amount of lymphocytic infiltration. Yellow bile pigment in the connective tissue. In places many eosinophiles and lymphocytes around transplant. A number of the lymphocytes seem to form plasma cells. Around the necrotic liver tissue some foreign body giant cells are produced. Grade 5.50.

6. *32 days.* Thirty-sixth generation of inbred rats. Kidney transplant. A great part necrotic; but quite a number of straight tubules in periphery of transplant and a few glomeruli preserved. There is a fair amount of lymphocytic infiltration; lymph vessels are filled with lymphocytes and these cells penetrate also into some living tubule cells. Also in periphery, fat and connective tissue are infiltrated with lymphocytes. Grade 5.

7. *32 days.* Thirty-sixth generation of inbred rats. Spleen transplant. Trabeculae with blood pigment cells; polygonal vacuolar, phagocytic cells, some of which take up blood pigment. Certain of these cells have much enlarged nuclei, especially those which penetrate into the necrotic material and take the latter up. Also megakaryocytes are found. In periphery of transplant some masses of lymphocytes. Grade 4.5.

COMMENT. The average grade in subseries I of Series B is 3 or 3.1 which is slightly below the average grade of the subseries I of Series A. As usual in various experiments the grades differ very much, the variations ranging between 5.6 and 1. Of special interest in this subseries is the behavior of various organs other than thyroid and cartilage. We notice a parallelism in the behavior of different tissues transplanted from the same donor to the same host. When thyroid has been destroyed, great parts of ovaries and uterus may be preserved, but they show very marked lymphocytic infiltration which may extend even to the follicles; in other cases, in which the homoio-reaction is still more severe, not only thyroid is replaced by fibrous tissue, but also uterus and ovaries; the interstitial tissue of the ovary is one of the last tissues to disappear. On the other hand, when

a germ epithelium cyst which is also much infiltrated. There are good large follicles, the theca interna of which shows some infiltration; but at some distance from the follicles there is a larger number of lymphocytes. Small to medium-sized good follicles and primordial follicles with ova; also atretic follicles. In connective tissue much infiltration with lymphocytes and lymph vessels are studded with these cells. Fallopian tubes and uterus also very much infiltrated. Grade 1.5.

2. *30 days.* Inbred rats of the 36th generation. Thyroid transplant destroyed; only fibrous tissue with small masses of lymphocytes. Uterus and ovary have been destroyed by fibrous tissue and some lymphocytes around vessels. Grade 1.

3. *25 days.* Inbred rats of the 37th generation. Instead of thyroid only fibrous tissue with some foreign bodies and foreign body giant cells visible and a very considerable lymphocytic infiltration in the fibrous tissue. Uterus transplant is replaced by fibrous tissue, with slight lymphocytic infiltration. Some interstitial tissue is the only remnant of ovary. Grade 1.

4. *37 days.* Thirty-seventh generation. Thyroid. A ring of acini with firm colloid is surrounded by a fibrous capsule; acinus cells of medium height. Loose connective and fat tissue and vessels in center, also some fibrous tissue and nests of squamous epithelium. Some large vessels penetrate through thyroid ring into center. Lymph vessels in center studded with lymphocytes; at poles of transplant and around the large vessels are masses of lymphocytes. In center of thyroid are many scattered cells of this type. Except for the presence of the lymphocytes the thyroid resembles an auto-transplant. The parathyroid is well preserved. In its periphery there are masses of lymphocytes around a vessel which penetrates somewhat into the parathyroid proper. Uteri and Fallopian tubes like autotransplants; epithelium, glands, connective tissue and muscle tissue are normal, but there are here and there small collections of lymphocytes. The ovarian transplant consists of a germ epithelium cyst, large good follicles with well developed granulosa containing many mitoses; primordial follicles with ova; also atretic follicles and interstitial tissue in various stages of development. In the fibrous tissue around transplant some masses of lymphocytes around vessels. Grade 5.

5. *32 days.* Thirty-sixth generation. Liver transplant. A great

probably by connective tissue cells as well. Fibrous tissue surrounding acini increased and much increase in central connective tissue. A plate of perichondrial cartilage lying at side of necrotic cartilage. In areolar and fat tissue around cartilage much fibrosis and many confluent fat cells; around blood vessels collections of lymphocytes. Bone surrounded by foreign body giant cells; bone marrow is not preserved. Apparently osteoblasts penetrate into necrotic cartilage, destroy it and form bone. Grade 2.75.

5. From first litter of 47th generation to second litter of 46th generation, 35 days. Grade 3.

6. From first litter of 47th generation to second litter of 46th generation, 35 days. In center of thyroid areolar tissue and a little fibrous tissue. Good ellipsoid of acini. In center epithelial pearls. Some lymph vessels filled with lymphocytes. At one pole of transplant considerable lymphocytic infiltration around vessels. Well preserved parathyroid. Large parts of cartilage also well preserved, surrounded by areolar and fat tissue with little lymphocytic infiltration and small amounts of connective tissue; but some newly formed fibrous tissue is found. Around necrotic cartilage plate of perichondrial cartilage. Tissue mostly free from lymphocytes and connective tissue.

COMMENT. The average grade of this subseries is 2.8, which is slightly less than the grade of subseries I. In strain A, the thyroid of second subseries is also below that of first subseries, but the difference between the grades of the two subseries is greater in Series A than in Series B. The average grade of Series B is 2.92, a figure slightly below that of Series A. The correspondence in the behavior of thyroid, parathyroid, cartilage and bone marrow in the different cases is very evident in these experiments; only when the reaction against thyroid and cartilage is slight are bone marrow partly preserved and megakaryocytes found. Grade 5.

SERIES C. TRANSPLANTATION FROM BROTHER TO BROTHER IN INBRED RATS

Subseries I. Rats of strain B of the 40th and 41st generation were used; eighteen experiments; pieces were taken out 25 to 40 days after transplantation. Grades were as follows: 1. *25 days.* Thyroid, parathyroid and cartilage. Grade 6. 2. *30 days.* Thyroid, cartilage,

thyroid and parathyroid are well preserved and show only a small amount of lymphocytic infiltration, uterus and ovary also are well preserved and are only slightly infiltrated. As one of us found previously in favorable cases, liver cells as well as bile ducts may be preserved; but also here lymphocytes begin to infiltrate the transplant and they may invade liver cells. Foreign body giant cells may form around the necrotic liver tissue. In favorable kidney transplants especially the straight tubules and glomeruli may be preserved, and in the spleen, trabeculae, megakaryocytes and perhaps some lymphocytic masses are found. Cells which invade and replace through phagocytosis the central necrotic part of the transplant may assume the character of vacuolar polygonal cells often possessing very large nuclei.

Subseries II, in which rats of strain 3 of the 46th and 47th generation were used. Six experiments constitute this subseries. Removal of transplanted pieces took place 25 and 35 days after transplantation.

The following grades were obtained: 1. From first litter 47th generation to third litter 46th generation, 35 days. Grade 1.

2. From first litter 47th generation to third litter 46th generation, 35 days. Thyroid with auto-structure, but much lymphocytic infiltration; lymph vessels studded with lymphocytes. In center and also in periphery a great deal of lymphocytic infiltration. Lymphocytes penetrate between and also in acini and destroy them. Strands of squamous epithelium in the central fibrous tissue also infiltrated with lymphocytes. A large number of acini still preserved and closely adjoining each other; some acini without colloid. Fibrous tissue surrounds cartilage and replaces areolar and fat tissue; lymph vessels studded with lymphocytes. There is also diffuse lymphocytic infiltration. Bone preserved, but around it is marked lymphocytic infiltration. Lymphocytes mingle with osteoblasts. Grade 3.5.

3. From first litter 47th generation to third litter 46th generation, 25 days. Grade 1.5.

4. From first litter 47th generation to third litter 46th generation, 25 days. In thyroid very intense lymphocytic infiltration. Lymph vessels studded with lymphocytes. Masses of these cells penetrate between and into acini and destroy them. Colloid in acini disappears partly through the activity of phagocytes. Many empty acini which are then compressed and invaded by lymphocytes and

chondrial cartilage proliferation. Trace of lymphocytes. 14. 25 days. Thyroid and cartilage. Grade 5. 15. 25 days. Thyroid, parathyroid and cartilage. Grade 6. 16. 41 days. Thyroid and cartilage. Grade 6. 17. 41 days. Thyroid and cartilage. Grade 6. 18. 41 days. Thyroid and cartilage. Grade 5.25. These six animals all belonged to the same litter.

COMMENT. The grades are 6 in seven experiments; 5.5 in two experiments; 5.25 in three experiments; 5, 4.75 and 4.5 each in one experiment; 4.25 in two experiments; 2.25 in one experiment. The average grade is 5.21. The rats used in these eighteen experiments belonged to five different litters; the results within the same litter differ according to the character of donor and host. The average strength of reaction is here much less marked than in the case of transplantation from one litter to the other.

Subseries II. Transplantations from brother to brother in inbred rats of strain A of the 42nd generation. Six experiments were made. Pieces were removed for examination at periods varying between 25 and 40 days after transplantation. The following grades and conditions were found: 1. 25 days. Grade 4. Thyroid and parathyroid on the whole well preserved, but marked lymphocytic infiltration especially at inner edge of thyroid ring. Lymphocytic masses destroy some tissue. In places connective tissue separates acini; but lymphocytes act independently of connective tissue. Cartilage on the whole well preserved. Some necrosis. In connective tissue around cartilage distinct lymphocytic infiltration.

2. 40 days. Grade 4.5. Condition of thyroid, parathyroid and cartilage similar to that in former experiments, but somewhat less lymphocytic infiltration; only slight infiltration between acini and only a few acini destroyed by lymphocytes. On the whole, well preserved cartilage. Some perichondrial proliferation in various places. Around perichondrium lymphocytic infiltration. Capillaries penetrate into necrotic part of cartilage. Where cartilage is thicker, it is necrotic *in toto*.

3. 25 days. Grade 1.5. Thyroid destroyed. Cartilage surrounded by fat and fibrous tissue. Lymphocytic infiltration of variable intensity. Some slight perichondrial proliferation. Connective tissue buds growing into necrotic cartilage. Animals from the same litter served for these three experiments.

4. 25 days. Grade 2.5. Thyroid infected. Cartilage transplant

uterus and ovaries. Grade 6. 3. 35 days. Cartilage and uterus. Grade 6. In the uterus transplant there were good papillae and mitoses in the unstriated muscle tissue; myxoid connective tissue separates epithelium from fibrous tissue. In one place some lymphocytic infiltration beneath epithelium. The muscle is hypertrophic. In this case transplantation had taken place into a sister which became pregnant subsequent to the transplantation; this explains probably the condition found in the uterus. 4. 40 days. Grade 4.5. Distinct lymphocytic infiltration. These four animals belong to the same litter. 5. 40 days. Uterus and ovaries. Grade 6. 6. 29 days. Grade 2.25. Thyroids with much fibrous tissue and large blood vessels in center; few acini are left. Each of the preserved acini is surrounded by fibrous tissue. Definite lymphocytic infiltration. Around cartilage marked lymphocytic infiltration. Also in fat tissue lymphocytic infiltration. Some regenerated perichondrial cartilage. 7. 35 days. Grade 5.25. Structure corresponds to autotransplant. Slight lymphocytic infiltration around thyroid and parathyroid. These three animals belong to the same litter.

8. 40 days. Grade 5.25. Thyroid and cartilage resemble autotransplants, except that at one pole of former there is marked lymphocytic infiltration; lymphocytes destroy here part of thyroid. 9. 30 days. Grade 5.5. Thyroid and cartilage resemble autotransplants except for slight collections of lymphocytes. Animals in last two experiments belong to the same litter.

10. 35 days. Grade 4.25. Thyroid and cartilage show structure of autotransplants, but now intense lymphocytic infiltration destroys part of thyroid and cartilage transplant contains a number of lymphocytes. 11. 25 days. Grade 4.25. Cartilage and uterus are on the whole well preserved, but show in places marked lymphocytic infiltration. In uterus much diffuse lymphocytic infiltration where vessels enter. At certain points marked infiltration of mucosa. 12. 35 days. Grade 5.5. Thyroid, parathyroid and cartilage like autotransplants, but a slight lymphocytic infiltration in places. Animals in last three cases belong to the same litter.

13. 25 days. Grade 4.75. Thyroid consisting of good ring of acini with much colloid; centre with considerable dense connective tissue. Extensive lymphocytic infiltration invading center of thyroid and part of parathyroid. Lymph vessels filled with lymphocytes. Cartilage surrounded by fat tissue. Around necrotic cartilage peri-

2. *45 days*. Grade 1.5. Thyroid and cartilage. 3. *40 days*. Grade 1.5. No thyroid remains. Cartilage preserved surrounded by fibrous tissue; moderate lymphocytic infiltration. Myxoid connective tissue and a few lymphocytes replace bone marrow. Regenerated perichondrial cartilage cells are again degenerating.

4. *40 days*. Grade 3.25. Thyroid and cartilage. Great parts of thyroid and parathyroid destroyed, but areas preserved in which acini are close together. Intense lymphocytic infiltration. In cartilage transplant fibrous tissue has replaced considerable amounts of fat tissue. Imperfect mantle of lymphocytes around cartilage; in fat tissue, small collections of lymphocytes. Some muscle fibers with chains of nuclei; lymphocytes between muscle fibers.

5. *45 days*. Grade 1.25. Thyroid and cartilage. 6. *45 days*. Grade 1.25. Thyroid and cartilage.

COMMENT. The average grade of this subseries is 2.04 which is considerably lower than the average grade of subseries I and II. Individual grades: 3.50; 1.50; 1.50; 3.25; 1.25; 1.25. Of interest again is the good correspondence in the conditions of different tissues or organs transplanted into the same host. Thus when the grade is low in the case of thyroid or parathyroid, bone marrow is replaced by myxoid connective tissue and lymphocytes. On the other hand, if a syngenesio-reaction is obtained with thyroid or cartilage transplants, muscle fibers with nuclear chains may be found as late as forty days after transplantation, with a grade of 3.25.

Subseries IV. This subseries corresponds to subseries III except that rats of strain B were used instead of strain A, the animals belonging to the 46th and 47th inbred generation. This subseries includes five experiments in which the transplantation was from brother to brother and two further experiments in which the transplantation was from mother to child. The grades were as follows: 1. *22 days*. Grade 4. Thyroid, parathyroid and cartilage. Auto-structure, but quite marked lymphocytic infiltration which has destroyed part of thyroid and parathyroid. Some slight increase in connective tissue in areolar and fat tissue around cartilage.

2. *35 days*. Grade 1.5. Thyroid, cartilage, bone and bone marrow. The latter has been replaced by myxoid connective tissue with some lymphocytes.

3. *35 days*. Grade 1.5. Thyroid and cartilage. Similar to experiment 2. No thyroid preserved; fibrous tissue around cartilage; some fat tissue preserved; some coalesced fat cells.

shows much connective tissue reaction and some lymphocytic infiltration. 5. 32 days. Grade 6. Thyroid, parathyroid and cartilage. The animals in these three experiments belonged to the same litter. 6. 25 days. Grade 2.5. No thyroid found. In fat tissue around cartilage, epithelial and giant cell reaction; confluent fat cells. Lymphocytic infiltration. Perichondrial proliferation and nodules of newly formed cartilage.

COMMENT. The average grade of this subseries is 3.5, which is a distinctly lower figure than that of the preceding subseries. We must of course consider that the number of experiments here is relatively small, so that the same importance cannot be attached to this low average grade as to the higher one in the preceding subseries. Of interest are the observations which confirm previous ones, demonstrating that the lymphocytic infiltration of transplants may be independent of proliferation of connective tissue and that blood vessels penetrate only into necrotic parts of this tissue. Again the identity of the reaction in transplantations in the same litter may show distinct differences in different experiments. The average grade of subseries I and II combined is 4.78.

Subseries III and IV. In these subseries, transplants were made from brother to brother of the 46th and 47th generations of inbred A and B rats. These experiments correspond therefore to subseries II in both Series A and Series B in which exchange of tissues in inbred rats occurred in animals belonging to different litters. In subseries II of Series A and B we found the average grade distinctly lower than in the corresponding subseries I in which a somewhat earlier generation of inbred rats were used. In a similar way we find in the case of brother to brother transplantations with animals of the 46th and 47th generations a distinctly lower grade than in subseries I and II of Series C in which animals of the 40th to 42nd generations were used for transplantation.

Subseries III. Brother to brother transplantations in strain A of inbred rats of the 46th and 47th generations. 1. 45 days. Grade 3.50. Thyroid, parathyroid and cartilage. Auto-structure. Intense lymphocytic infiltration has destroyed great parts of thyroid. Lymphocytes penetrate parathyroid also in direction from periphery toward center. Cartilage transplant in places shows some marked infiltration and some increase in connective tissue; some confluent fat cells.

plantations would give an indication as to the changes in the individuality differentials which the long continued inbreeding through brother to sister matings has produced. We therefore carried out a series of transplantations of this nature. In both of these series we used animals of the 37th and 38th generation (subseries I) as well as from the 46th and 47th generation (subseries II).

I. *Transplantation from strain A to strain B. Subseries I.* Thirty-seventh and 38th generation. Fourteen cases comprise this subseries. One case in which the piece was removed as early as six days after transplantation cannot be graded, although we can state that even at this early period there was a definite reaction against the transplant on the part of the host. In the other cases the time of removal of the pieces varied between 19 and 45 days. In the majority of cases thyroid (sometimes with parathyroid) and cartilage were used for transplantation; in other cases ovary and uterus with or without thyroid. The grades were as follows: 24 days, 1; 24 days, 1; 26 days, 2 (?); 25 days, 5; 26 days, 4 (?); 29 days, 5.50; 23 days, 2; 19 days, 3; 30 days, 2.50; 45 days, 1; 36 days, 2.5; 24 days, 4; 31 days, 1. Average grade 2.65.

The following example may be cited: Strain A to strain B, 37th generation, 36 days. Thyroid has been destroyed; only fibrous tissue is found. In the transplanted ovary a germ epithelium cyst is found including papillary structures resembling the fimbria. Good follicles, medullary ducts and interstitial tissue, also some unstriated muscle tissue seen. We find here a very distinct lymphocytic infiltration in the ovary proper as well as in the surrounding tissue; some infiltration is seen also in the epithelium of the Fallopian tube and there is a considerable amount in the unstriated muscle. The lymph vessels are filled with lymphocytes. Grade 2.5.

COMMENT. The average grade of the reactions in this subseries is very similar to that found previously by one of us in the case of homoiotransplantation in non-inbred rats, where the average grade varied between 2.5 and 2.7. We find again a variation in the intensity of the reaction in the individual cases. The example which we cite brings out very well that the ovaries and Fallopian tubes are more resistant to the action of the homoio-toxins than the thyroid; but notwithstanding this absolute difference there is a correspondence between the reaction, inasmuch as in the ovary as well as in the Fallopian tube the lymphocytic reaction is quite marked in this case,

4. 40 days. Grade 5.75. Thyroid, cartilage and bone marrow. Thyroid and cartilage well preserved with surrounding fat tissue; very slight collection of lymphocytes in thyroid. Bone marrow partly well preserved with megakaryocytes.

5. 40 days. Grade 3.75. Thyroid, parathyroid and cartilage. Thyroid with auto-structure. Intense lymphocytic infiltration in thyroid and parathyroid; only remnants of these tissues remain. Strands of lymphocytes penetrate parathyroid and collect in center. Some good areas of thyroid left, where acini are close together and contain colloid; but lymphocytes penetrate also these parts entering between and into acini. Well preserved cartilage, surrounded by fat tissue in which fibrous tissue is increased. Many small strands of lymphocytes in areolar and fat tissue.

6. 25 days. Grade 1.5. From mother of 46th to child of 47th generation. Thyroid and cartilage.

7. 25 days. Grade 4. (The same litters as 6). Thyroid, parathyroid and cartilage. Auto-structure of thyroid; but increased connective tissue and lymphocytes; many acini destroyed. Also in fat tissue around cartilage some increase in connective tissue and marked lymphocytic infiltration.

COMMENT. The individual grades in this subseries are 4; 1.5; 1; 5.75; 3.75; 1.5; 4. The average grade is 3.1. As observed before, the grade is lower in the later than in the earlier generation. Of interest again is the correspondence in the behavior of various organs transplanted into the same host. When thyroid, parathyroid and cartilage transplants are well preserved, bone marrow is likewise preserved, while in cases in which the reaction against these other tissues is severe the bone marrow is replaced by connective tissue. The average grade of subseries III and IV is 2.63 as compared with the average grade 4.78 of subseries I and II in which the earlier generation of rats was used. The average grade of all the brother to brother transplantations is 4.03.

SERIES D. CROSS-TRANSPLANTATIONS FROM STRAIN A TO STRAIN B AND FROM STRAIN B TO STRAIN A

The proper control experiments for transplantation within the inbred strains would be transplantations from animals belonging to strain A to animals belonging to strain B and *vice versa*; such trans-

lymphocytic infiltration. Where new formation of connective tissue takes place around the cartilage, hemorrhage is often found.

COMMENT. The average grade is in this subseries about the same as in the first subseries; there is therefore no definite difference between the earlier and later generations; such as we find in the transplantation within the inbred strains A and B. Again the result agrees with that obtained in ordinary homoiotransplantation. In the first case which we cite, it is interesting to note that with reactions which stand at the border-line between syngenesio--and homoio-reaction, striated muscle tissue can show certain regenerative phenomena, although lymphocytes invade the transplanted muscle. In the second case we find, with a typical homoio-reaction, myxoid fibrillar bone marrow, a condition which confirms our previous findings.

II. *Transplantation from strain B to strain A. Subseries I.* Thirty-seventh and 38th generation. Sixteen experiments were carried out; the time of examination varied between 19 and 50 days. Thyroid, parathyroid, cartilage, ovary and uterus were the principal organs or tissues used for transplantation. The grades in the different experiments are as follows: 19 days, 1.75; 19 days, 1; 23 days, 2.75; 28 days, 2; 19 days, 1.5; 28 days, 4; 25 days, 3 (?); 35 days, 1.5; 36 days, 2; 25 days, 3; 23 days, 3; 23 days, 3.5; 37 days, 5; 50 days, 5; 50 days, 2; 39 days, 1. The average grade is 2.6.

Some examples which show special points of interest may be cited.

1. 25 days. Thyroid transplant consists mainly of fibrous tissue in which are situated compressed acini without colloid. The fibrous tissue surrounds bundles of these compressed acini. Lymphocytes collect around the collapsed acini, penetrate into them and replace them. Lymph vessels are filled with lymphocytes. The transplanted ovary consists of a germ epithelium cyst, in part of which the germ epithelium has been lost. In such places connective tissue proliferates into the cyst cavity. Small follicles are preserved, at least their granulosa is present, but the eggs have disappeared in some cases; there is lymphocytic infiltration around these follicles as well as in the interstitial tissue. Fallopian tubes, either with or without epithelium; there is lymphocytic infiltration. Much fibrous tissue in these transplants. Grade 3.

2. 36 days. Thyroid has been destroyed; cartilage is surrounded by lymphocytic masses. The ovarian transplant again consists of a germ epithelium cyst, the epithelial lining of which has not been

although as a rule lymphocytes are rather small in number in the ovarian transplants.

Subseries II. Forty-sixth and 47th generation. Eight cases. In all except in one case, in which the removal of the piece took place after 17 days, the pieces were removed at a period varying between 20 and 30 days. The grades of only seven cases are given, as in one case the grade was uncertain: 22 days, 4.25; 30 days, 3; 30 days, 1.50; 30 days, 3; 25 days, 2.75; 20 days, 1; 20 days, 2.25. Two examples may be cited in which in addition to thyroid (with or without parathyroid) and cartilage, bone and bone marrow or striated muscle were transplanted.

1. 25 days. Forty-sixth generation. Thyroid transplant with a large amount of fibrous tissue in center; much lymphocytic infiltration. A great part of the peripheral ring of acini is destroyed; the remaining acini lose their colloid, they collapse and lymphocytes enter and destroy them. Parathyroid also much infiltrated with lymphocytes. Lymph vessels studded with lymphocytes. Cartilage is on the whole preserved. Regeneration of the perichondrium leads to the formation of a cartilage plate and regenerated perichondrial cartilage penetrates also into necrotic cartilage. In the fat tissue around cartilage much increase in connective tissue and much lymphocytic infiltration in places. At one end of cartilage there are striated muscle fibers with nuclear chains, and some lymphocytic infiltration between them; but as usual there is less lymphocytic infiltration here than thyroid. Grade 2.75.

2. 20 days. Forty-sixth generation. The thyroid transplant consists now mainly of hyaline fibrous tissue. In the periphery of this fibrous tissue there are a number of acini, mostly compressed and without colloid, a few still containing colloid. Fibroblasts migrate around the acini and form fibrous bands; lymphocytes penetrate between and into the collapsed acini and destroy them. Some lymph vessels in the fibrous tissue filled with lymphocytes. Around the cartilage transplant there is much fibrous tissue; also lymphocytic infiltration is seen. Into the thick necrotic cartilage, connective tissue with lymphocytes is penetrating from the side. Areolar and fat tissue is partly replaced by fibrous tissue. Instead of bone marrow we find fibrillar or myxoid connective tissue with some lymphocytes. Few osteoblasts are left; only peripheral bone is preserved; the center is necrotic. On the whole, there is here not much

only incompletely and ova may be lost. There is definite lymphocytic infiltration in the ovary. Of interest is the fact that when the epithelial lining of the germ epithelium cyst has been lost, the connective tissue grows into the cyst. In these transplants also the epithelium exerts a restraining influence on the underlying connective tissue. The second case cited shows likewise a better preservation of the ovary than of the thyroid; but the ovary is not completely preserved as it would have been with a more favorable character of the individuality differentials and there is much lymphocytic infiltration. It is somewhat better preserved and less infiltrated with lymphocytes in the third case, in which the thyroid is similar to the first case, but in the surrounding fibrous tissue there is also in this instance marked lymphocytic infiltration. In the fourth case, we find thyroid, parathyroid and uterus much better preserved; the ovary, however, shows about the same structure as in the first, second and third cases, but the lymphocytic infiltration is here distinctly less. The ovary is therefore not as fine a reagent for differences in the individuality differentials as is the thyroid, but the activity of the lymphocytes gives also in the ovary, to a certain extent at least, a measure of the similarity or dissimilarity of the individuality differentials. In the fifth case, however, the injury of the ovarian tissue and especially of the uterus has progressed much further.

Subseries II. This subseries consists of only three experiments; the rats belonged to the 46th and 47th generation. Thyroid, parathyroid and cartilage were used for transplantation. The grades were as follows: 30 days, 2; 30 days, 1.25; 22 days, 3. Average grade 2.1.

In the first experiment the thyroid is so densely infiltrated with lymphocytes that it resembles a lymph gland; parathyroid tissue alone remains. Cartilage is well preserved. A great amount of fibrous tissue replaces the areolar and fat tissue, but part is left. Very dense mantle of lymphocytes around cartilage and in fat tissue; lymph vessels filled with lymphocytes. Bone marrow is replaced by fibrillar connective tissue with lymphocytes. Grade 2.

COMMENT. The second subseries does not differ in any essential respect from the other subseries in this group of experiments. On account of the small number of experiments, not much importance can be attached to a slight lowering of the grade (2.1 as compared to

completely preserved, owing to desquamation. There are some follicles with granulosa epithelium and some atretic follicles. Much lymphocytic infiltration. Grade 2.

3. *25 days.* This specimen shows conditions similar to specimen 1. The thyroid is compressed through much development of fibrous tissue. Acini are without colloid and are much infiltrated with lymphocytes, the lymph vessels being studded with these cells. In other places connective tissue cells grow around compressed acini and there is little lymphocytic infiltration; but in general the lymphocytes destroy the tissue over considerable areas. Ovaries show small and large good follicles, but again in some small follicles the ova have disappeared. The granulosa cells proliferate by mitoses. There is a germ epithelium cyst; neither in granulosa nor in the interior is there much lymphocytic infiltration. In uterus unstriated muscle tissue is preserved. There is some but not a dense lymphocytic infiltration; however, the lymph vessels in the uterus are studded with lymphocytes and the connective tissue of the transplant is much infiltrated. Grade 3.

4. *37 days.* Thyroid well preserved; large acini with high epithelium and good colloid. Large vessels in center; around the vessels lymphocytic masses and also around center some collections of lymphocytes. The structure is that of autotransplant. Parathyroid also well preserved. In uterine epithelium, mitoses. The mucosa is cellular and fibrillar and well formed muscle layer in uterus. Some fibrous tissue around uterus; only slight lymphocytic infiltration. Ovary with germ epithelium cyst; small follicles and atretic large follicles; some small isolated lymphocytic masses. Grade 5. In a fifth specimen with grade 2 examined after 50 days there is in the ovary a large follicle without good ovum, a germ epithelium cyst, and interstitial tissue. In the connective tissue of the transplant there is a very marked lymphocytic infiltration, while the uterus transplant has been invaded, destroyed and replaced by dense masses of lymphocytes.

COMMENT. The grade in this subseries is about the same as the grade with the reciprocal subseries strain A to strain B, 2.65 in the latter and 2.6 in the former. Where there is a marked reaction in the case of the thyroid, we find a correspondingly marked reaction in the case of the ovary and tube, although relatively the latter transplants are better preserved. In the ovaries the follicles develop

reactions. On the other hand, such tissues as liver and also spleen and bone marrow are very sensitive while thyroid and parathyroid hold a somewhat intermediate position; the latter organs are therefore the best indicators of the intensity of reaction on the part of the host. But while these differences in absolute intensities of reaction between tissues and organs exist, there is an unmistakable correspondence in the relative intensities in reaction found if into the same host different tissues or organs from the same donor are transplanted. Of course, there is in addition a difference in the resistance of the various constituents of the same organ. These observations which one of us has made in previous investigations were again confirmed in these experiments.

If we compare first the grades in ordinary homoiotransplantation with the grades obtained in transplantation from inbred strain A to inbred strain B or *vice versa*, we find in the latter the average grade is 2.57, while in the former the grades are 2.5 in the first and 2.7 in the second series. This indicates practical identity in the breadth of variation in the individuality differential in ordinary homoiotransplantation and in transplantation from strain A to B or *vice versa*. This result might be expected inasmuch as while many generations ago the ancestors of strains A and B belonged to the same litter they have been kept separate through many generations of inbreeding restricted to each strain. If we now compare the average grades of transplants within the inbred strains A and B with those from strain A to strain B or *vice versa* or with the grades of ordinary homoiotransplants, we find that the former are slightly higher, but the differences are relatively so small that they may come within the range of error. Thus the average grade for transplantation within strain B is 2.92, while the average grade for homoiotransplantation in series II is 2.7. This would indicate that through long continued inbreeding it is not possible to produce a decidedly greater similarity between the individuality differentials than is found in the case of ordinary white rats. This conclusion is confirmed in comparing the average grades from brother to brother transplantation in ordinary rats and in inbred rats. In ordinary rats the average grade is 4.66 to 4.75, while in inbred rats (strain A to B combined) it is 4.03. In both cases the average similarity between the individuality differentials of brothers is much greater than that between ordinary rats not belonging to the same litter. Again the averages in the grades

2.6 in the first subseries). The example cited shows well the correspondence in the reaction towards thyroid and cartilage; in both transplants the lymphocytic infiltration is very pronounced. As to the whole group in series D, the total average grade of transplantation from strain A to strain B is 2.61, while the total average of transplantation from strain B to strain A is 2.53. These two figures are about the same. The total grade of both kinds of transplantations, namely from strain A to B and B to A is 2.57 which corresponds very closely to the grade of ordinary homoiotransplantation among non-inbred white rats; here the average grade of thyroid transplants is approximately 2.6. Transplantations from strain A to strain B and *vice versa* represent therefore typical homoiotransplantations in groups of white rats which do not represent different varieties (as would for instance white, cream and hooded rats). There is no marked difference in this group of experiments between the results obtained with animals of the 37th and 38th generation on the one hand and with animals of the 46th and 47th generation on the other hand, although the average grade in the later generation is slightly lower; but the difference between subseries I and II is so small that it probably falls within the range of probable error.

DISCUSSION AND CONCLUSIONS

In reviewing this series of transplantation as a whole, the outstanding result established is the great difference which exists between transplantation in inbred rats and inbred guinea-pigs. In the latter we had found a very marked effect of inbreeding on the individuality differential, while in the case of the inbred rats, this is either absent altogether or is very slight indeed. A comparison between the average grades obtained in non-inbred families and in inbred families of rats is of interest in this connection. However, before comparing these figures, it is well to recall that in grading the intensity of reactions against various tissues and organs there are distinct differences in the absolute intensities of reaction; thus ovarian transplants are on the whole better preserved than, for instance, thyroid transplants and in the former the lymphocytic reaction is rather mild; uterus also is relatively resistant, although the lymphocytic reaction may be intense. Cartilage itself is very resistant, but the surrounding fat and areolar tissue shows distinct

individuality differentials the strongest individuals were in each case those in which the individuality differentials were most dissimilar. Though this assumption seems plausible, yet in view of the behavior of the most recent inbred generations of rats in which the reactions were especially severe, we must take into consideration the possibility that in addition other, so far unknown, factors may come into play.

SUMMARY

1. We have analyzed in these investigations in the rat the effect of long continued inbreeding by means of successive brother and sister matings on the constitution of the individuality differentials in inbred strain A and inbred strain B and we compared the results obtained with those obtained previously in various kinds of homoio-, syngenesio- and in autotransplantation in non-inbred rats. In order to be able to compare the results in the various series, we used the same system of grades which we had previously employed in our investigation in non-inbred guinea-pigs and rats and subsequently also in inbred guinea-pigs. The following list of the most important average grades obtained in the various series of experiments give the main results obtained by us.

Non-inbred rats: Autotransplantation, 6. Homoiotransplantation, Series I, 2.7; Series III (transplantation into different varieties), approaching 1.

Inbred rats: Exchange of tissues between animals belonging to different litters. *Strain A*. Subseries I (37th and 38th generations), 3.4. Subseries II (46th and 47th generations), 1.12. Total average for strain A, 3. *Strain B*. Subseries I (37th and 38th generations), 3.0 (3.1). Subseries II (46th and 47th generations), 2.8. Total average for strain B, 2.92. Total average grade of strains A and B slightly below 2.96. Transplantation from strain A to strain B; 2.61. Transplantation from strain B to strain A, 2.53.

Non-inbred rats: Brother to brother transplantation, 4.66 to 4.70.

Inbred rats: Brother to brother transplantation, strain A and B, 40 to 42 generations, 4.78. Brother to brother transplantation, strain A and B, 46th to 47th generations, 2.63. Total average of brother to brother transplantations, 4.03.

2. We may conclude from these results that inbreeding of rats through successive brother and sister matings through a range ex-

of both inbred and non-inbred rats belong to the same order but this time the average grade of brother to brother transplantation in non-inbred families happens to be slightly higher than in inbred families. We may again, therefore, conclude that the long continued inbreeding through successive brother and sister matings does not result in a greater homogeneity of the individuality differentials of the various members of the same inbred strain.

If we compare the average grades in the 37th to 40th generation with the grades in the 46th and 47th generation we find some interesting differences. Thus in Series A and B the figures for the former are 3.5 and 3.0 and for the latter 1.12 and 2.8 respectively. In brother to brother transplantations within the inbred strains the average grade for the former is 4.78 and for the latter 2.63. This would indicate that instead of decreasing the intensity of the reactions on the part of the host against the transplants, as might have been expected, on the contrary an increase in the intensity of the reaction took place the longer the inbreeding continued. But in appraising these differences we must consider the fact that the number of experiments carried out with the later generations is as yet rather small, and additional experiments are necessary to determine whether this difference is real.

However this latter point may be decided, it is certain that through long continued inbreeding in rats no appreciable approach to homogeneity of the individuality differentials, such as was to a certain extent accomplished in the guinea-pigs, has been reached. This result was not foreseen; as a matter of fact the literature cited on this subject led one to expect the opposite result. As to the causes of differences of behavior of inbred rats and guinea-pigs, one might consider the fact that while in the guinea-pig the mating between brothers and sisters was of a non-selective character, in the case of the rat the strongest individuals were chosen. Thus in the rat a marked deterioration of the stock was prevented, while in the guinea-pig there were definite indications of a deterioration in the characteristics of the inbred animals. Coincidentally with deterioration in stock in the case of inbred guinea-pigs the individuality differentials become more and more similar, while with the preservation of the full vigor of the stock in the case of inbred rats the individuality differentials remains dissimilar. We may assume that while the weakest members of the family showed the greatest similarity between the

tending between 37th and 47th generations does not lead to a distinct diminution in the intensity of the reaction against the transplant if transplantation occurs within the inbred families; at most there may perhaps be a very slight diminution. This holds good also for brother to brother transplantations; while the reactions in these latter experiments are much less marked than in the case of ordinary homoiotransplantations, they are at least as severe in the inbred as in the non-inbred rats.

3. These data indicate that in contrast to the results obtained in inbred guinea-pigs, where inbreeding led to a marked decrease in the intensity of the reaction and where the individuality differentials became very similar (although not yet identical through inbreeding), in the rat successive brother and sister matings, continued through many generations, do not lead to a marked increase in the similarity of the individuality differentials in the inbred animals.

4. This result may be due to selection of the strongest individuals of a litter for mating, the individuality differentials being perhaps most dissimilar in constitution in the strongest individuals. There is, however, the possibility that other not recognized conditions may help to bring about this result. This is at least suggested by the greater severity of the reaction against the transplants in the later as compared with the earlier generations of inbred rats. Further experiments must be made to determine whether this increased intensity in reaction is a constant phenomenon, or is merely accidental.

5. Our previous observations as to the differences in the severity of the reactions against different kinds of tissues and organs were confirmed in this series; we also confirmed our former conclusion that while the absolute severity of the reactions differs according to the relations between the individuality differentials of host and transplant, the relative severity is the same in all the organs tested so far and depends mainly upon the character of the individuality differentials.

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The fusiform bacillus is a large, pleomorphic, often irregularly staining, gram-negative bacillus with pointed ends. According to Keilty⁵ and Glynn⁷ it is encountered in practically every mouth. Tunnicliff⁹ believes the fusiform bacillus and the associated spirillum to be different stages in the life cycle of the same organism. This view is not in accordance with the observations of Krumwiede¹⁰ and others.

Streptococci. Almost all investigators agree that hemolytic streptococci are rarely associated with the lesions of pyorrhea alveolaris. In connection with the study of *Streptococcus viridans* in relation to periodontal infections the investigations of Goadby,¹¹ Gilmer and Moody,¹² Hartzell and Henrici¹³ are worthy of careful consideration. Goadby established rather conclusively, it seems, the rôle of dental infections in the etiology of arthritis deformans. Gilmer and Moody found a hemolytic streptococcus in acute alveolar abscesses and *Strep. viridans* in the chronic. Hartzell and Henrici conclude that streptococci belonging to the viridans group are constantly present in periodontal infections. In no case did they encounter hemolytic streptococci. They report 29 strains of *Strep. viridans*, 14 of which belong to *Strep. mitis* group, 10 *Strep. salivarius* and 1 *Strep. fecalis* (Andrewes and Horder),¹⁴ the remaining 4 being unclassified at that time. Price¹⁵ reports, in sixty-seven successive cases showing periodontal lesions, the finding of many types of streptococci and that *Strep. fecalis* forms 65.5 per cent of these different types. These conclusions differ widely from those of other workers.

MATERIAL AND METHODS

Direct Smears. Material was obtained from 201 unselected cases of clinically positive pyorrhea occurring in patients at the Victoria Hospital and the Ontario Hospital, London, Ontario.

With a wooden applicator, sharpened to a thin chisel-like end and sterilized, material was obtained in each case from the space (gingival) between the gum and the root of the tooth showing the greatest involvement. In most instances this was the labial surface of incisors. The smears were air-dried, gently fixed in the flame and then stained by the method employed by Bass and Johns.² While this stain gives rather poor differentiation of the various constituents of the ameba cell, it stains bacteria and pus cells well. Thionin re-

PYORRHEA ALVEOLARIS: THE RÔLE OF CERTAIN MICROÖRGANISMS FOUND IN THE LESIONS*

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This investigation was undertaken to study the probable significance of *Endamoeba gingivalis*, spirilla, *Bacillus fusiformis* and streptococci found in the lesions of pyorrhea alveolaris. Direct smears were examined in 201 unselected cases of pyorrhea and in seventeen control cases showing normal-appearing gums and teeth, free from pyorrhea. Cultures were made from thirty cases of pyorrhea and also from the seventeen control cases.

LITERATURE

The literature concerning bacterial factors in pyorrhea is extensive. This report will refer only briefly to some of the more recent investigations.

Amebas. *Endamoeba gingivalis* has been the subject of investigation by Smith and Barrett,¹ Bass and Johns,² Sandford and New,³ Hecker,⁴ Keilty,⁵ Mitchell, Culpepper and Ayer⁶ and others. There is insufficient evidence, it seems from these studies, to establish the pathogenicity of the ameba. Glynn⁷ summarizes the evidence against the pathogenicity of this ameba. However, Bass and Johns² believed it to be the specific cause of pyorrhea alveolaris. Keilty⁵ describes a particular type of gingivitis, apparently of infrequent occurrence, in which, he believes, the *Endamoeba gingivalis* is the exciting cause.

Spirilla and B. fusiformis. The term spirilla is used in this investigation to include all types of spirilla and spirochetes observed in the mouth cavity. The present classification of spiral forms is made largely on the basis of morphology and motility. Noguchi⁸ has described a mucin-producing spirochete which he isolated from pyorrhea lesions and to which he attributes, in part, the strong fetid odor of pyorrhea. Glynn⁷ regards the rôle of spirochetes in pyorrhea lesions as still an open question.

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RESULTS AND OBSERVATIONS

The Occurrence of Amebas, Spirilla and Fusiform Bacilli. In the 201 cases diagnosed as pyorrhea alveolaris, 192 (95.5 per cent) showed the presence of amebas. These are shown in Fig. 1. All positive cases examined showed the presence of *B. fusiformis* and spirilla (Fig. 2) in large numbers and pus cells (Fig. 3) were invariably present, usually in large numbers. The spirilla are of more than one type. They vary greatly in number, width and depth of their curves and in length. This variation is well shown in Fig. 4.

In the series of seventeen clinically normal gums, amebas were absent in all cases. *B. fusiformis* and spirilla, while in much smaller numbers than in the pyorrhea cases, were present in all of the seventeen normal gums.

Cultures from Pyorrhea Cases. The accompanying table shows the results of the cultures for streptococci in pyorrhea cases. In these thirty cases it will be seen that in every case green-producing streptococci (*Streptococcus viridans* [Schottmüller]) were present. *Strep. viridans* was present in large numbers and in all cases it formed decidedly the majority of organisms present on the plates. In several instances the cultures on blood agar plates, obtained as above described, showed a pure growth of *Strep. viridans*. From the fermentation reactions these belong to two groups namely *Strep. mitis* and *Strep. salivarius* (Andrewes and Horder), nineteen of which are *Strep. mitis* and eleven *Strep. salivarius*. Examination of the accompanying table will readily show the subgroups (according to Brown) to which these belong and the number in each subgroup. In no instance were hemolytic streptococci present.

In no case was *Strep. fecalis* found while Price¹⁵ reports that 65.5 per cent of the different types of streptococci, found by him in periodontal lesions, belong to the *Strep. fecalis* group. He also reports the finding of *Strep. hemolyticus* as well as *Strep. viridans*. In these two important respects the results of this study differ from those of Price.

All the cultures made on rabbit blood agar for the hemolytic influenza bacillus failed to show the presence of this organism.

Cultures from Normal Controls. In the seventeen cultures obtained from the gums of the normal controls *Strep. viridans* was present in only six instances. Of the remaining eleven cultures, ten

commended by Dupray¹⁶ as a diagnostic stain in pyorrhea proved unsatisfactory as did also Wright's blood stain. The stained smears were carefully examined for amebas, *B. fusiformis*, spirilla and pus cells. The identity of the amebas was further established in a number of cases by warm-stage examination of fresh pyorrheal pus in normal saline. In several instances dark-field illuminations of fresh material were made to study the morphology of the spirilla and their activity. Direct smears from a number of cases were further studied by a special silver nitrate method which Gilbert and Bartels¹⁷ used in the examination of dry smears for *Treponema pallidum*. I have obtained excellent preparations by this method applied to the staining of spirilla in dry smears from pyorrhea lesions.

Cultures from Pyorrhea Cases. The determination of streptococci in pyorrhea lesions was made by cultural methods in thirty cases. For this purpose aerobic blood agar plates, made with plain meat infusion agar, adjusted to pH 7.6, plus 5 per cent defibrinated sheep blood, were used. The selected tooth together with its adjacent gum, buccal mucous membrane and immediate surrounding area was thoroughly cleansed with sterile applicator and cotton and 50 per cent alcohol. Wherever possible labial surfaces of lower incisors were used because in this region saliva did not run down and contaminate the field. Under careful precautions material was obtained from a deep portion of the lesion by means of the sterile sharpened applicator, previously mentioned. This material was immediately planted on a blood agar plate by parallel streaks on the surface. From this plate suspected streptococcus colonies were picked to blood agar slants for identification and further study. After twenty-four hours' incubation, the fermentation reactions of the isolated strains were tested on the carbohydrates—lactose, mannite, salicin, saccharose, raffinose and inulin and they were grouped and subgrouped according to Brown.¹⁸ To differentiate between *Strept. viridans* and the pneumococcus, likewise a green-producer, a bile solubility test was done in all cases. Later in the investigation, several cultures were made from pyorrhea lesions on rabbit blood agar for hemolytic influenza bacilli.

Control Cases. As controls, direct smears and cultures were made and studied by technic similar to that employed in clinically positive cases, from seventeen young medical students with normal-appearing gums and teeth free from pyorrhea.

positive cocci arranged in pairs and short chains. These have the morphology of the streptococcus.

DISCUSSION

Significance of Amebas, Spirilla and Fusiform Bacilli. It is probable that the ameba should be regarded as nonpathogenic and harmless. In so far as has been observed it does not invade living peridental tissues. When present in pyorrhea pus it occurs in relatively very small numbers as compared with the pus cells and bacteria present. It seems to be an expression of an unclean mouth, the necrotic material and debris being the habitat of the ameba, because in this series they do not occur in clean normal mouths. In my experience the more unclean the mouth the greater is the probability of finding the ameba. All animal experiments carried out by other investigators with this ameba have failed to produce pathologic lesions.

From a study of the literature it appears that spirilla and fusiform bacilli have received little recognition as important etiologic factors in pyorrhea alveolaris. Kline²⁰ has reported seven cases of spirochetal pulmonary gangrene associated with severe dental caries as a primary focus and referred to seventeen other cases reported in the literature. As previously stated, spirilla and fusiform bacilli occurred in all normal cases examined as well as in pyorrhea cases, they do not invade living peridental tissues and they occur in large numbers in smears obtained superficially. It may, therefore, be concluded that they are probably of little pathologic significance in pyorrhea.

Rôle of Streptococcus viridans in Pyorrhea Lesions. The pathogenicity of the *Strep. viridans* group is well known and has been established by workers mentioned in this paper and by others. In this study *Strep. viridans* was constantly present in pyorrhea lesions and absent in eleven of seventeen normal gums. It may be that some of the supposedly normal cases showing *Strep. viridans* are in reality early incipient cases of pyorrhea which, at this stage, are not recognizable clinically. This organism is a constant inhabitant of the normal buccal cavity. Zinsser,²¹ in discussing the bacteria in the normal mouth and pharynx makes the following statement: "Of the streptococci the Viridans is almost always present. The isolation of a 'viridans' from inflammatory processes of the mouth

showed no growth on the blood agar plates after seventy-two hours incubation while the eleventh showed only two colonies both of which were organisms other than *Strep. viridans*. All of the six cultures, showing *Strep. viridans*, presented a striking diminution in the number of streptococci as compared with those cultures from pyorrhea cases while one of these six showed one lone streptococcus colony. Of the six strains of *Strep. viridans* isolated in this normal

TABLE SHOWING THE RESULTS OF DEEP CULTURES FROM GINGIVAL SPACE
FOR STREPTOCOCCI. THIRTY CASES OF PYORRHEA ALVEOLARIS AND
SEVENTEEN NORMAL CONTROL CASES

	Groups of <i>Strep. viridans</i>	Subgroups (according to Brown ¹⁸)				
		.1	.2	.3	.4	Total
Positive pyorrhea	<i>Strep. mitis</i>	8	5	5	1	19
	<i>Strep. salivarius</i>	5	5	0	1	11
*Normal gums	<i>Strep. mitis</i>					0
	<i>Strep. salivarius</i>	2	4	0	0	6

* 11 of the 17 cultures from normal gums showed no streptococci.

series all belonged to the group *Strep. salivarius*. (For subgroups see table.) In no case of this series was *Strep. hemolyticus* encountered.

Sections of Teeth and Gums from Pyorrhea Cases. Specimens of teeth, gums, alveolar process and adjacent jaw showing well marked pyorrhea lesions, in a number of cases, were removed at necropsy and decalcified. From these, sections were prepared and stained by hematoxylin and eosin and by MacCallum's technic¹⁹ for bacteria in tissues. From careful examination of these sections it is seen that the ameba does not invade the tissues of the gum. Throughout the stroma of the gum adjacent to the tooth involved, there is a well marked chronic inflammatory reaction with a preponderance of plasma cells (Fig. 5). In the sections stained with MacCallum's stain there are large numbers of spirilla, fusiform bacilli and gram-positive cocci situated rather superficially in the necrotic material and debris in the gingival space. However, situated deeper, in the peridental membrane beyond the necrotic margin, are a few gram-

In the greater number of cases more than one type of *Strep. viridans* may be present in individual cultures from pyorrhea lesions, although I have found that this is not always the case. If two or more types be present, usually one type is decidedly predominating. The results here reported were determined by fishing from each culture a representative of the predominating type of *Strep. viridans* colony.

The pathogenicity of different strains of *Strep. viridans* isolated from pyorrhea lesions was tested upon rabbits with varying results. Some strains were apparently nonpathogenic. Others killed the animals within twenty-four hours. One strain of *Strep. mitis* produced a fatal vegetative endocarditis, strikingly similar to that found in human cases dying of *Strep. viridans* endocarditis.

In a number of cases I have found that the blood serum of patients showing well marked pyorrhea lesions contains agglutinins for the predominating strain of *Strep. viridans* found in their lesions.

Further observations are now in progress upon variations in the types of *Strep. viridans* encountered in individual cases, pathogenicity, toxicity and agglutination reactions. These will be reported later if results seem to justify publication.

SUMMARY AND CONCLUSIONS

1. *Endamoeba gingivalis* was present in direct smears in 95.5 per cent of 201 pyorrhea cases examined. Evidence is here submitted in support of the belief that this ameba is a harmless parasite. It was not found in the clean, normal gums examined.

2. *B. fusiformis* and spirilla were found in the smears from all of the cases of pyorrhea alveolaris studied and were also present in each of the seventeen normal gums. For reasons here given they are probably of little significance in the production of the pyorrhea lesion.

3. In making cultures a special technic was employed in an attempt to exclude contamination from saliva, buccal mucosa, adjacent teeth, gums and other sources.

4. *Strep. viridans* was present in each of thirty cases of pyorrhea examined culturally. In all instances it was decidedly the predominating organism present on the blood agar plates. The isolated strains belong to two groups, *Strep. mitis* and *Strep. salivarius* respectively, and seven subgroups (table). In seventeen normal gums

and throat, therefore, has very little true significance unless it is isolated from a closed process, such as a tooth abscess, or unless other strong corroborative evidence can be adduced." However, fully recognizing the significance of this statement, by the technic employed in this investigation I believe that I have excluded contamination from the surrounding surfaces. The fact that ten of the seventeen cultures from normal controls showed no growth on aerobic blood agar plates seems to indicate the relative efficiency of the technic. With the thorough preliminary cleansing and by the method previously described, material obtained under these circumstances from a deep portion of the pyorrhea lesion would be almost equivalent to that obtained from a closed process. It is important in cultures obtained in this manner to take into consideration relative numbers, if different types are present. In pathologic lesions it is usually logical to attach importance to the predominating type. It is interesting to note that 78 per cent of the streptococci present in normal saliva are of the *Strep. salivarius* type (Glynn ⁷) while 63 per cent of the *Strep. viridans* strains in my series isolated from pyorrhea cases belong to the *Strep. mitis* group.

The constant association of *Strep. viridans* in large and predominating numbers with pyorrhea, as shown by cultural methods, its absence in the majority of normal gums cultured and the occurrence, in sections of pyorrheal tissue, of a gram-positive coccus morphologically a streptococcus in the deeper peridental tissues, may be taken as evidence in favor of this organism being of much importance in pyorrhea.

That the production of pyorrhea alveolaris should be due to a single factor seems highly improbable. It may be that a combination of factors, such as nutritional disturbances, traumatic influences (mechanical and chemical) and increasing age, initiates the condition and a bacterial infection follows. Since pyorrhea alveolaris is a suppurative lesion, *Strep. viridans*, essentially a nonpyogenic coccus, is not likely the exciting cause of this pathologic condition. However the constant presence, in large numbers, of *Strep. viridans* in deep portions of the lesion, its known pathogenicity, its invariably constituting the majority of organisms in cultures on blood agar plates and its invasion of living peridental tissues, present evidence that it is a grave menace in a primary focus for the production of certain systemic diseases.

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DESCRIPTION OF PLATE

PLATE 56

- FIG. 1. Three amebas (*Endamoeba gingivalis*, Gros) stained with carbol fuchsin and methylene blue. The dark inclusion-bodies and vacuoles are clearly shown. The nucleus is poorly defined with this strain. $\times 1000$.
- FIG. 2. Spirilla and fusiform bacilli stained with carbol fuchsin and methylene blue. Note the bands of irregular staining in the fusiform bacilli. $\times 1000$.
- FIG. 3. Polymorphonuclear leucocytes. This shows the acute suppurative nature of the pyorrhea lesion and gives an idea of the large numbers of pus cells encountered. $\times 1000$.
- FIG. 4. Spirilla stained by Fontana's method. Different types of spirilla demonstrating the variation in morphology are here shown. $\times 1200$.
- FIG. 5. Hematoxylin-eosin section of decalcified tooth and gum showing well marked pyorrhea alveolaris. This section shows a pronounced chronic inflammatory reaction with plasma cells predominating and occasional polymorphonuclear leucocytes. The dentine appears at the extreme left of the figure. The clear area is the gingival space. $\times 650$.

NOTE: — All figures are photomicrographs of material obtained from pyorrhea cases. Figs. 1 to 4 inclusive, Zeiss apochromatic 2 mm. objective, compensating $\times 10$ ocular. Fig. 5, 4 mm. objective, $\times 10$ ocular. Wratten M plates were used for all figures.

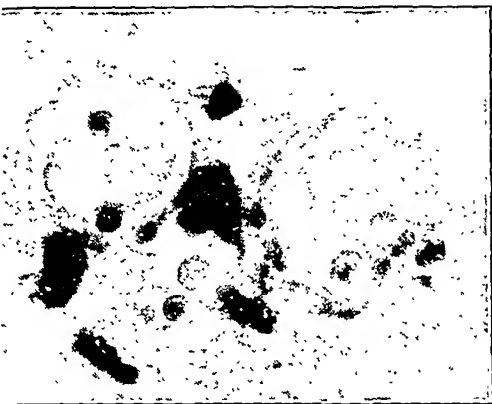
studied *Strep. viridans* was absent in eleven. Strains of this organism in the remaining six belong to one group, namely *Strep. salivarius* of which there are two subgroups (table). For reasons stated, *Strep. viridans* is probably of much importance in pyorrhea.

5. Hemolytic streptococci were not present in any of the cases examined culturally nor were hemolytic influenza bacilli.

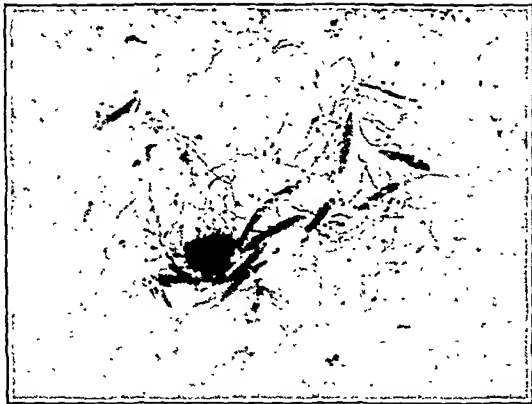
The author takes pleasure in expressing his indebtedness to Professor H. H. Bullard for valuable advice and kindly criticism. He wishes also to express his sincere appreciation of the kind coöperation of Dr. Robinson and his staff at the Ontario Hospital and of the staff at Victoria Hospital in furnishing material which has made possible this study.

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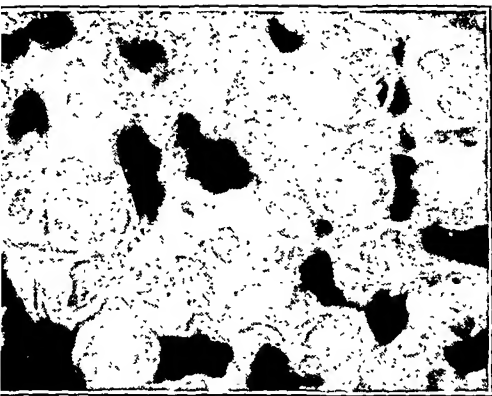
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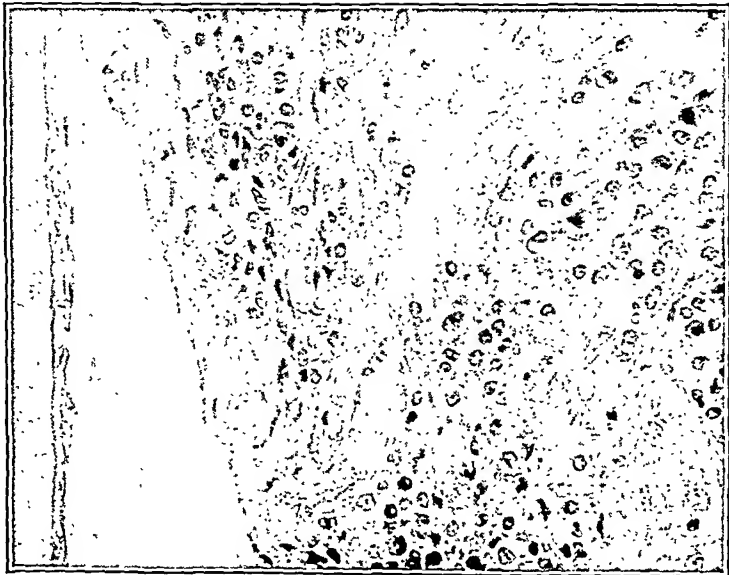
2



3



4



5

reversed, and begins to clear up first in the tibia. Thus the marrow of the tibia, which may be involved only rather late, is, nevertheless, a more sensitive index of the extent and degree of the pathologic process than is, for instance, the marrow of the femur. Bearing in mind all the limitations that one must accept in examining small specimens of marrow from the tibia, there yet remains much to be learned from them, and this is especially true when two or more specimens can be obtained from a single case at different periods in the course of the disease.

TECHNIC OF TIBIAL PUNCTURE

The operation of tibial puncture must, of course, be carried out under the strictest surgical precautions. I am greatly indebted to Dr. Robert C. Cochrane for all the specimens of bone marrow obtained during life. The soft tissues over the middle of the anterior or mesial aspect of the tibia were carefully infiltrated with a 2 per cent novocain solution over an area sufficient to permit a longitudinal incision about 4 cm. in length down to the periosteum. After elevation of the periosteum, the marrow cavity was entered by means of a small drill or a trephine which removed a 6 mm. cylinder of bone. Then small pieces of the marrow, 1 to 3 mm. in diameter, were removed by means of a small sharp bone curette, the cavity of which was rather well recessed and straight-sided. The specimens were immediately fixed in Zenker's solution, later embedded in paraffin, sectioned, and stained with eosin and methylene blue. Hemorrhage into the tissues sometimes complicated the interpretation of the histology. In sewing up the wound it was found best to use no sutures except in the skin, and to apply a tight bandage to prevent oozing. No untoward results have been observed in a series of eighteen operations, and the fact that the discomfort to the patient was not great is indicated by there having been two tibial punctures on several patients and three punctures on one patient. It is appropriate, however, to express deep appreciation for the coöperation of the patients who willingly submitted to these operations.

The description of the clinical cases and the observations on the condition of the bone marrow follow. The nomenclature of cells in the bone marrow is according to the terminology established by Sabin.²

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THE PATHOLOGY OF THE BONE MARROW IN PERNICIOUS ANEMIA *

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In spite of the enormous amount of attention that has been devoted to the subject, it must be admitted that very little is actually known about the pathogenesis of pernicious anemia. Cohnheim,¹ who, in 1876, was the first to describe the bone marrow, regarded the anemia as due to a primary disturbance of blood formation, and many authorities have since maintained the same point of view. Another conception of the disease, however, regards the bone marrow changes as of a compensatory nature, and as the result of an attempt on the part of the organism to make good the losses arising from excessive blood destruction. This theory has become the more generally accepted one, even though the evidence of increased blood destruction is not convincing. Recent investigations of pernicious anemia have been, for the most part, concerned with the cell types and chemical constituents of the peripheral blood, but there is reason to believe that in order to understand the pathology of this disease, and indeed that of the other diseases of the blood-forming organs, more attention must be paid to the bone marrow than is now customary among clinical hematologists. The present study is a contribution to the pathology of the bone marrow in pernicious anemia, and the observations indicate that the changes in the blood are largely the result, rather than the cause, of abnormal bone marrow function.

There are two main reasons why comparatively few attempts have been made to correlate the pathologic changes in the bone marrow

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c.mm.; leucocytes, 1200 per c.mm.; serum bilirubin, 0.57 mg. per 100 cc. On July 22, a second tibial puncture was performed, and it is of interest, that shortly afterwards a spontaneous remission set in.

Biopsy of tibial marrow, July 22, 1925. The tissue was very similar to that obtained on May 18, but there were occasional cells containing globules of fat, and there was a definite increase in the number of normoblasts and mature red blood corpuscles. The megaloblastic hyperplasia was still the predominant feature, however, and there were many mitoses of megaloblasts. There were also more cells of the leucocyte series. The venous sinusoids remained compressed and obscure.

On August 7, the hemoglobin was 34 per cent, and the erythrocyte count was 1.5 million per c.mm. On September 2, "great clinical improvement" was noted. On September 28, the hemoglobin was 52 per cent and the red blood cell count was 2.4 million per c.mm. During the following months the patient lived on a diet containing large amounts of liver, and a striking clinical remission took place. On March 12, 1926, the blood examinations were as follows: hemoglobin, 86 per cent; red blood cell count, 5.5 million per c.mm.; leucocytes, 6200 per c.mm.

Biopsy of tibial marrow, March 12, 1926. A large part of the specimen was composed of fat cells, and was without evidence of myeloid hyperplasia. The intersinusoidal capillaries in this area were filled with normal erythrocytes, but it was impossible to determine whether this was a true hyperemia or was the result of hemorrhage during the operation. Another part of the specimen consisted of well filled fat cells, separated from one another by small groups of myeloid cells which apparently were within intersinusoidal capillaries. The capillary endothelium was moderately hypertrophic and hyperplastic. Within the capillaries were a few megaloblasts and erythroblasts, very many normoblasts and many mature erythrocytes. There was also a moderate number of cells of the leucocyte series. The venous sinusoids were widely distended with blood, and the conical openings of the intersinusoidal capillaries into them were easily made out in many places. The whole picture resembled that of the early stage of simple marrow hyperplasia.⁹ Fig. 5 shows the general character of the more cellular part of the specimen, and Fig. 7 shows the predominance of normoblasts in the islands of myeloid cells.

CASE 1. A. F., a man 33 years old, with a history typical of pernicious anemia extending over two years, and apparently in his third relapse. Physical examination, laboratory tests and hematologic studies were all characteristic of the disease.

May 18, 1925, examinations of blood showed: hemoglobin, 15 per cent; red blood cells, 0.6 million per c.mm.; leucocytes, 5400 per c.mm.; serum bilirubin, 1.5 mg. per 100 cc.

Biopsy of tibial marrow, May 19, 1925. The tissue was very cellular, and the fat, which is normally present in the marrow of the tibia, had been completely replaced by cells. The histologic picture was as complex as that of the bone marrow obtained postmortem in pernicious anemia, and was, in general, similar to it in character. The most striking feature was the enormous hyperplasia of megaloblasts. These cells, which have large vesicular nuclei with a definite chromatin network, and basophilic cytoplasm, were found in clumps, cords and columns, and sometimes as separate cells. Some of this separation of individual cells was unquestionably due to shrinkage in the process of fixation. For the most part, the megaloblasts were adherent to one another and the appearance of the tissue was suggestive of a tumor. Rapid multiplication of megaloblasts was indicated by the great number of mitoses, sometimes as many as six or eight being found in a single field with the oil immersion lens. Fig. 1 is a photograph under low power to show the general character of the tissue and the distribution of the megaloblastic hyperplasia. Fig. 2 is a photograph under high magnification, and Figs. 3 and 4 are drawings to illustrate the megaloblasts and their mitoses. In addition to the megaloblasts, the marrow contained many normoblasts and cells intermediary between megaloblasts and normoblasts. There were relatively few mature erythrocytes. Giant cells were present in moderate number, while myelocytes and leucocytes were fairly common. A few myelocytes were found in mitosis. In contrast to the usual findings in bone marrow obtained postmortem from cases of pernicious anemia, there was scarcely any phagocytosis of red blood cells by clasmatoctes.⁸ The venous sinusoids, which are frequently such a prominent feature in early or slight degrees of bone marrow hyperplasia, were narrow, compressed and recognized only with great difficulty.

Two months later, on July 20, 1925, the blood examinations were as follows: hemoglobin, 13 per cent; red blood cells, 0.8 million per

began to live on a diet containing much liver, and a prompt clinical remission set in with rapid rise in the red blood corpuscles. On April 29, 1926, the erythrocytes had risen to 3.5 million per c.mm., and a second biopsy was performed on the tibial marrow. The other blood examinations showed: leucocytes, 8500 per c.mm.; serum bilirubin, 0.18 mg. per 100 cc.

Biopsy of tibial marrow, April 29, 1926. The tissue consisted largely of cells well filled with fat. It had considerably more fat than has normal vertebral marrow. Fig. 6 is a photograph, under low magnification, to show the relation of fat to cellular areas (compare with Fig. 8). The small cellular areas between the fat globules were chiefly composed of erythrocytes in the intersinusoidal capillaries, but there were also a great many normoblasts, some of which showed definite mitoses. The normoblasts were often in large clumps which filled the spaces between the fat cells, as shown in Fig. 9. Megaloblasts were not a prominent feature but there were a few small groups and a few scattered single cells. Mitosis were rare among the megaloblasts. Leucocytes and giant cells were few in number. The venous sinusoids were outlined by pigment in the endothelium, and they were comparatively wide and distinct.

Two months later, on June 29, 1926, the hemoglobin had risen to 92 per cent; and the erythrocyte count to 4.9 million per c.mm.

Summary of findings in Case 2

During a severe relapse (March 12, 1925), the fat of the bone marrow was almost entirely replaced by myeloid cells, and there was a striking hyperplasia of megaloblasts with many showing mitoses. Many normoblasts were present, but mature red blood cells were not particularly numerous. Early in the development of a rapid remission (April 29, 1926), the bone marrow was characterized by a great increase in fat deposits and by large numbers of normoblasts and mature erythrocytes, but at this time, only a few megaloblasts were observed. The venous sinusoids were much more distended and distinct during the remission than during the relapse.

CASE 3. V. D., a man 48 years old, with symptoms of anemia for one year, together with the physical and laboratory findings typical of pernicious anemia. On July 15, 1925, the hemoglobin was 43 per cent, and the red blood cell count 2.4 million per c.mm. On August

Summary of findings in Case 1

At the height of a severe relapse (May 19, 1925), the marrow was characterized chiefly by the complete replacement of fat by myeloid cells, and by the great hyperplasia of megaloblastic tissue with numerous mitoses. There were many normoblasts, but relatively few mature erythrocytes, and the venous sinusoids were narrow and compressed. Two months later (July 22, 1925), just before the onset of a clinical remission, the marrow was similar except for the presence of a few cells containing fat, and a relative increase of normoblasts and mature red blood cells. Ten months after the first examination (March 12, 1926), during a remission in which the erythrocyte count was normal, the cellular hyperplasia had almost completely disappeared and the marrow consisted largely of fat cells. In the small capillary spaces, between some of the fat cells, were many erythrocytes and normoblasts, but the more primitive cells (megaloblasts) were comparatively few in number. The venous sinusoids had become widely distended with blood.

CASE 2. C. H., a woman 46 years of age, who had been under observation for four years as a typical case of pernicious anemia. There had been several relapses followed by periods of moderate remission. On March 12, 1926, she was in the hospital during a severe relapse and the blood examinations were as follows: hemoglobin, 24 per cent; erythrocytes, 0.9 million per c.mm.; leucocytes, 4400 per c.mm.; serum bilirubin, 0.9 mg. per 100 cc.

Biopsy of tibial marrow, March 12, 1926. The tissue showed a great hyperplasia of myeloid cells and relatively few cells containing fat globules. Fig. 8 is a photograph to show the general character of the tissue, and the displacement of the fat. The histology resembled that seen in Case 1 during the relapse. There was a striking hyperplasia of megaloblasts with a general tendency on the part of the cells to adhere to one another and to lie in clumps and columns. Rapid cell growth was indicated by the many mitoses seen in the megaloblasts. There were many normoblasts, but few mature red blood cells, few giant cells and few leucocytes. The venous sinusoids were often outlined by pigment granules in the endothelium, but they were compressed and indistinct. Abnormal phagocytosis of erythrocytes or of pigment was not observed.

Immediately after the above observation was made, the patient

Summary of findings in Case 3

Two specimens of tibial marrow were obtained during the course of the prolonged terminal relapse, the first about six months and the second about one month before death. The two specimens resembled each other closely and both contained a considerable amount of fat. In this connection it may be noted, however, that sections from other cases show that even at death the replacement of fat by myeloid hyperplasia is frequently much less complete in the tibia than in the femur. The marrow from both biopsies showed great hyperplasia of megaloblasts, but this process was somewhat more extensive in the second specimen. Normoblasts were present in great numbers, but there were comparatively few mature erythrocytes in either piece of tissue.

CASE 4. B. C., a woman who gave her age as 48 years, but who appeared to be considerably older. She had been "weak" for four months, and reported having numbness of the fingers for one month. Physical examination and laboratory tests were typical of pernicious anemia. Blood examinations were as follows: Aug. 1, 1925, hemoglobin, 18 per cent; erythrocytes, 0.8 million per c.mm.; leucocytes, 5500 per c.mm.; August 20, hemoglobin, 25 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 4800 per c.mm.; August 31, hemoglobin, 42 per cent; erythrocytes, 2.3 million per c.mm.; leucocytes, 6900 per c.mm.; September 30, hemoglobin, 63 per cent; erythrocytes, 3.5 million per c.mm.; leucocytes, 8100 per c.mm. A biopsy performed on September 2 was, therefore, done at a time when the patient was making rapid spontaneous improvement.

Biopsy of tibial marrow, September 2, 1925. The fat of the marrow had been entirely displaced and the tissue had a solid appearance. Mature red blood cells were the most prominent feature, and it is possible that they may have been due to hemorrhage, but their relation to the islands of myeloid tissue suggests that they were true components of the marrow. Megaloblasts were found in a limited number of small clumps and as scattered individual cells. Mitosis of megaloblasts was infrequent. There were a great many normoblasts in large and small groups. Giant cells were few in number, and leucocytes were fairly numerous. The sinusoids were compressed.

20, the hemoglobin was 31 per cent, and the red blood cell count 1.0 million per c.mm. On September 2, the blood examinations showed an erythrocyte count of 1.1 million per c.mm.; leucocytes, 4100 per c.mm.; reticulocytes, 1.1 per cent; and the serum bilirubin, 0.8 mg. per 100 cc. The patient was thus having a relapse.

Biopsy of tibial marrow, September 2, 1925. The tissue contained a considerable amount of fat, about the amount found in normal vertebral marrow. The general character is shown in Fig. 11. There were many rather large islands of megaloblasts arranged in clumps or columns, and many single megaloblasts. Megaloblasts and erythroblasts were frequently in close relation to the fat cells, and their position suggested that they arose from the endothelium lining the intersinusoidal capillary spaces. Mitoses of megaloblasts were found in moderate numbers. There were very many normoblasts, and several definite mitoses were observed among them. Mature erythrocytes were present in moderate numbers. Phagocytosis of red cells was not seen. Giant cells were rare, and there were few myelocytes or leucocytes. The sinusoids were compressed and indistinct.

In the subsequent months there was no striking change in the patient's condition, but he gradually failed in health. On Jan. 18, 1926, the blood examinations were as follows: hemoglobin, 24 per cent; erythrocytes, 0.9 million per c.mm.; leucocytes, 3500 per c.mm.; serum bilirubin, 0.66 mg. per 100 cc. On this day a second tibial biopsy was performed. The patient left the hospital soon after, and died at home on Feb. 26, 1926. The second biopsy was thus made almost at the end of the terminal relapse.

Biopsy of tibial marrow, January 18, 1926. The tissue contained about as much fat as was present in the first biopsy. There was a striking hyperplasia of the megaloblasts, rather more than in the first biopsy, the cells lying in clumps which were so large that they sometimes filled the spaces between the fat cells. Fig. 10 shows the amount of fat present and the extensive hyperplasia of megaloblasts. Mitoses were common among the megaloblasts, as many as five having been observed in a single field of the oil immersion lens. There were very many normoblasts but comparatively few mature red blood cells. For the rest, the tissue was similar to that observed on September 2.

as normal marrow. Unfortunately no specimen was obtained during the more complete remission that took place eight months later.

CASE 5. J. S., a man 61 years old, who had run a characteristic course of pernicious anemia for about eighteen months, most of which time he had been under observation. In August, 1925, he entered the hospital, shortly after the onset of his second relapse, with physical examination and laboratory tests wholly typical of pernicious anemia. The results of blood examinations were as follows: March 26, 1925, hemoglobin, 72 per cent; erythrocytes, 3.9 million per c.mm.; leucocytes, 8200 per c.mm.; June 12, 1925, hemoglobin, 82 per cent; erythrocytes, 3.3 million per c.mm.; leucocytes, 5800 per c.mm.; Aug. 25, 1925, hemoglobin, 30 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 5500 per c.mm.; Sept. 2, 1925, erythrocytes, 1.3 million per c.mm.; leucocytes, 4900 per c.mm. During these months he was, therefore, slowly going into a severe relapse.

Biopsy of tibial marrow, September 2, 1925. A part of the section consisted of fat cells with mature erythrocytes filling the inter-sinusoidal capillary spaces (hemorrhages?). The rest of the section showed much hyperplasia of myeloid cells between the fat cells, (there was about as much fat here as in normal vertebral marrow) and in one area the fat was completely displaced. The hyperplastic areas were composed chiefly of megaloblasts, growing in columns and islands, and often filling the space between the fat cells. There were many mitoses of megaloblasts. Fig. 12 shows a clump of megaloblasts between fat cells and one megaloblast undergoing division. Just below and to the left, there is another megaloblast in the same phase of mitosis, but it does not show clearly in this focus. There were a great many normoblasts, a moderate number of mature red blood cells, and many cells of the leucocyte series. The sinusoids were compressed and indistinct.

In the subsequent weeks the patient continued to fail gradually. The blood examinations on Dec. 7, 1925, were as follows: erythrocytes, 0.7 million per c.mm.; leucocytes, 9200 per c.mm.; serum bilirubin, 1.21 mg. per 100 cc. He died on the same day.

Tibial marrow at necropsy (two hours after death). The tissue showed the typical, extremely confused structure usually found at necropsy. The fat had been almost entirely replaced by myeloid

On Oct. 27, 1925, the blood examinations were as follows: hemoglobin, 71 per cent; red blood cells, 3.5 million per c.mm.; leucocytes, 9100 per c.mm. The second biopsy was performed on Oct. 28, 1925, at a time of marked clinical improvement, and just before the patient left the hospital. She was then in a rather prolonged remission of moderate degree. The patient was subsequently fed on liberal amounts of liver, and about eight months later the erythrocyte count was 4.6 million per c.mm.

Biopsy of tibial marrow, October 28, 1925. This tissue was taken from somewhat higher up in the tibia than was the first specimen. It contained about as much fat as normal vertebral marrow. There were a few small clumps of megaloblasts and scattered individual cells, but they were not a prominent feature. Mitoses of megaloblasts were found rarely. There were many normoblasts, but only a moderate number of mature erythrocytes. Cells of the leucocyte series were very numerous and giant cells were not uncommon. The sinusoids were somewhat more distinct than in the tissue from the former biopsy. The appearance of the tissue resembled that of normal active marrow from a vertebra, except that there were rather more megaloblasts.

Summary of findings in Case 4

The tissue obtained on Sept. 2, 1925, soon after the onset of a spontaneous improvement, contained no fat, and the cells consisted largely of erythrocytes and normoblasts. Megaloblastic hyperplasia was not a prominent feature. The specimen is to be compared with that of Case 2, taken on April 29, 1925, which was also obtained during a remission, and which differs from Case 4 chiefly in that it contained more fat. Both sections showed a predominance of normoblasts and mature red blood cells, and in both the megaloblastic hyperplasia was relatively slight. Two months later (Oct. 29, 1925), after the patient's still further improvement, the bone marrow showed an increase of fat, and a cell picture which resembled that of normal active vertebral marrow except for the moderate increase of megaloblasts. There were no longer such large numbers of erythrocytes in the marrow and it is probable that they had passed out into the capillaries. It may also be that after the relapse was over, the part of the marrow near the epiphysis (this specimen was taken from high up on the tibia) continued to function

Biopsy of tibial marrow, February 12, 1926. The specimen consisted of fat tissue and showed no evidence of increased vascularity or cellular hyperplasia. It was normal, atrophic, fatty marrow.

Summary of findings in Case 6

This specimen was taken shortly after the onset of what subsequently proved to be a very rapid remission following a first and relatively short relapse. If this sample represents the general character of the tibial marrow, then the presence of a normal fatty marrow can be explained either by the fact that, at least in the first relapse, the pathologic process does not necessarily extend to the marrow of the tibia, or by the assumption that the pathologic process can disappear completely, and very rapidly, during a period of clinical improvement.

CASE 7. A. H., a woman 64 years old, with a history of pernicious anemia of two years duration. She entered the hospital during a relapse with an erythrocyte count of about 1.0 million per c.mm. For three weeks she ate considerable amounts of liver, and at the end of this time (May 26, 1926) the blood examinations were as follows: hemoglobin, 57 per cent; erythrocytes, 2.6 million per c.mm.; leucocytes, 6000 per c.mm.; reticulocytes, 4.4 per cent.

Biopsy of tibial marrow, May 26, 1926. The specimen showed essentially a fatty, normal, aplastic, tibial marrow. There were a few rather large endothelial cells with vesicular nuclei, lying between the fat cells and forming the walls of intersinusoidal capillaries. These resemble the hypertrophied endothelial cells which are characteristic of the earliest stage of marrow hyperplasia,⁹ but they were so few in number that it is impossible to say that they were not within the normal limits.

Summary of findings in Case 7

In this case, as in Case 6, it is unfortunate that no biopsy was performed before the onset of the remission. Here again, it cannot be determined whether the normal appearing marrow was the result of a rapid clearing up of the pathologic process after the onset of a remission, or whether it merely indicated that even after the disease had lasted two years, the tibial marrow had not become involved. The fact that in Case 2 a remission of even greater degree and

cells. The prominent feature was the hyperplasia of megaloblasts, the cells being single, in clumps, or in columns. There were many mitoses among the megaloblasts. Normoblasts were common. Giant cells were rare. There were many myelocytes and leucocytes. Throughout the tissue, and so numerous that there were often six or eight in a high power field, were clasmatoocytes (endothelial cells) which had phagocytosed erythrocytes, normoblasts, and occasionally leucocytes. The number of ingested red blood cells was enormous. The red cells within the phagocytes usually retained their normal appearance, and there were few phagocytes containing hemosiderin. Some of the sinusoids were broad and well defined, but most of them were compressed and difficult to distinguish.

Summary of findings in Case 5

On Sept. 2, 1925, during a severe relapse which took place three months before death, the bone marrow contained a considerable amount of fat, but between the fat cells there was an active hyperplasia of megaloblasts, with many normoblasts. On Dec. 7, 1925, the bone marrow, taken two hours after death, showed such an extensive increase in myeloid cells that the fat had almost completely disappeared. Hyperplasia of megaloblasts was the predominant feature, but there were many normoblasts and cells of the leucocyte series. Of considerable interest was the appearance of great numbers of clasmatoocytes which had phagocytosed erythrocytes and normoblasts.

In addition to the above five cases in which two or more specimens of bone marrow have been obtained at different times, brief mention will be made of two additional cases in which only one specimen has been obtained, but from which, nevertheless, certain impressions may be formed.

CASE 6. M. H., a man 55 years old, came under observation in the first relapse of typical pernicious anemia. The symptoms had lasted about six months. Blood examinations on Jan. 25, 1926, were as follows: hemoglobin, 50 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 2040 per c.mm.; reticulocytes, 1.6 per cent. Liver feeding was begun on January 28, and on February 12 the blood examinations showed: hemoglobin, 50 per cent; erythrocytes, 1.8 million per c.mm.; leucocytes, 3900 per c.mm.; reticulocytes, 9.1 per cent.

but many are found in the stages of most active hyperplasia, while giant cells are almost always abnormally decreased.

The study of bone marrow obtained by biopsy at different stages of pernicious anemia throws light on the relation of the pathologic process in the bone marrow to the clinical course of the disease, and the observation of Zadek⁶ that the marrow hyperplasia disappears during clinical remissions has been confirmed by this investigation. In general, the more active the disease and the more profound the relapse, the greater is the pathologic hyperplasia of the bone marrow. This is, to some extent, indicated by the relation of cellular hyperplasia to the amount of fat in the marrow. Thus in the terminal stage, as shown in tissue obtained at necropsy, there is usually a complete or nearly complete replacement of fat by myeloid hyperplasia (see, for instance, Case 5). A similar but sometimes less marked condition is found during a serious relapse. In a severe relapse, Case 1 showed complete disappearance of fat, and the marrow in Case 2 contained extremely little fat. On the other hand, considerable fat may be present at autopsy in the marrow of a peripheral bone like the tibia, and in Case 5, the marrow contained a good deal of fat during the progress of the terminal relapse. The first specimen in Case 4, obtained after the onset of a remission, contained no fat. The relationship is thus by no means constant, and the displacement of fat, although an index of the cellularity of bone marrow, does not necessarily run parallel to the clinical course of the disease.

Of much greater significance in relation to the clinical course of the disease than either the amount of fat or the degree of cellularity of the bone marrow are the types of cells of which the marrow hyperplasia is composed. Thus the evidence indicates that severe relapses are characterized by a predominance of rapidly proliferating megaloblasts, while in remissions or during periods of clinical improvement the megaloblastic hyperplasia becomes less evident, and more mature cells of the red blood cell series, normoblasts and erythrocytes, become the prominent feature in the marrow. Essentially the same observations were made by Zadek.⁶ Cases 1 and 2 show the change in cell type very clearly, for specimens of bone marrow were obtained, first in relapse and then at the height of a remission, or during the development of it. The first specimen from Case 4, taken soon after the onset of a remission, showed many normoblasts and erythrocytes,

rapidity was accompanied by a striking change, but not by a disappearance of the pathologic process, may be taken to suggest that in Cases 6 and 7 the marrow of the tibia had never been affected.

DISCUSSION

The essential lesion of the bone marrow in pernicious anemia, and that which dominates the histologic picture during clinical relapse, is an hyperplasia of the myeloid cells in which the megaloblasts play the chief part. The development of the process can be studied in a simple fatty marrow like that of the tibia more easily than in complex active marrow like that of the vertebrae, but the lesion seems to be the same wherever it occurs. The megaloblasts develop from the endothelial cells of the intersinusoidal capillaries which, in an atrophic marrow, are collapsed and almost invisible between the fat cells.^{2, 9} They are formed within the lumen of the capillary, and where active proliferation is taking place, the capillary may be entirely filled by one, two or more rows of megaloblasts. This is illustrated in Fig. 12. In the more active stages of the pathologic process, as seen at necropsy, and in tissue taken at biopsy during a relapse, the proliferation of megaloblasts is very rapid. This is indicated by the extraordinary number of mitoses, and by the tendency of the cells to remain adherent to one another in columns and clumps, rather than to separate off as individual cells. Coincident with the hyperplasia of megaloblasts there is also a limited development of the more highly differentiated forms of the red blood cell series, namely erythroblasts, normoblasts, and erythrocytes. These cells also proliferate in the capillary spaces between the fat cells, and, since the marrow is confined within a rigid shaft of bone, their multiplication goes hand in hand with a disappearance of the globules of fat. It is evident, however, that even after the globules of fat are displaced, the fat cells remain in their normal position in the marrow, for specimens taken at biopsy show that when the myeloid hyperplasia recedes, the fat cells take up globules of fat again, and fill the space of the marrow cavity. The fat cells are practically invisible during the period of myeloid hyperplasia, but they are a constant element in the structure of the marrow, and serve an important subsidiary function by compensating for the proliferation and retrogression of the true blood-forming cells. The number and types of leucocytes vary in the bone marrow in pernicious anemia,

the previous hyperplasia. In Case 2, there was a striking decrease in megaloblastic hyperplasia early in the course of a rapidly developing remission. In Case 4, the marrow taken soon after the onset of a remission showed little megaloblastic hyperplasia. On the other hand, Case 5 illustrates that as a relapse progresses the opposite condition will be found, namely that the megaloblastic hyperplasia increases.

Such histologic evidence, however, does not prove that the pathologic condition of the marrow is the cause of the anemia, in spite of the fact that it suggests that the decrease in megaloblastic hyperplasia precedes the improvement in the hematologic picture. It is, therefore, interesting to correlate what is known about the pathologic histology of the marrow with some of the characteristic changes of the red blood corpuscles in the peripheral blood. In a severe relapse, with an erythrocyte count of 1.0 million or less per c.mm., the number of young cells in the blood, as shown by a count of reticulocytes, is usually relatively increased, but absolutely normal or decreased. Thus a reticulocyte count of 2 per cent of the total red blood cells, which is common under such circumstances, actually means that no more young cells are being put out by the bone marrow per day than is the case in a normal person with a red blood cell count of 5.0 million per c.mm. and approximately 0.5 per cent reticulocytes. This is so in spite of the fact that the active bone marrow, in the patient with a relapse of pernicious anemia, is an organ many times larger than that in the normal subject. The extensive hyperplastic marrow delivers fewer young cells in a unit of time than a normal marrow. There is cellular hyperplasia with functional inefficiency. This becomes particularly clear if one compares the situation with that in congenital hemolytic jaundice, a disease which is probably of a primary hemolytic nature. Here the marrow continues for months and years to put out so many young cells that the percentage of reticulocytes may be 15 to 30 or more of a red blood cell count between 4.0 and 5.0 million per c.mm. In addition to this, it is during the development of a remission that one often finds the large numbers of reticulocytes in the peripheral blood in pernicious anemia, and it has been seen that at exactly this period, when the bone marrow is beginning to function more effectively, the megaloblastic hyperplasia is beginning to disappear. Such histologic and hematologic evidence, therefore, indicates that

but comparatively few megaloblasts. In Case 3, on the other hand, there was a slight increase in megaloblastic hyperplasia as the relapse progressed, and in Case 5 the marked hyperplasia of megaloblasts, seen during the course of the terminal relapse, was found to be still further increased at necropsy. Although the megaloblastic hyperplasia seems to be the essential feature of the pathology of the bone marrow in pernicious anemia, it cannot be stated that the lesion is necessarily specific for this disease.

Cases 6 and 7, in which normal, fatty marrows were obtained early in the development of clinical remissions, suggest, without definite proof, that pernicious anemia may exist for a considerable time and even present the picture of a serious relapse, without involvement of the marrow of the tibia. It is quite possible that for indefinite periods the disease may be limited to those parts of the marrow that are normally active. At necropsy an involvement of the marrow of the femur is, of course, practically constant.

The study of specimens of bone marrow taken at different stages in the course of pernicious anemia also furnishes evidence on the long disputed question of whether the anemia is primarily due to an increased destruction of red corpuscles, or whether it is the result of a primary disorder of blood formation in the bone marrow. At present the most widespread opinion appears to be that the disease is a hemolytic type of anemia, and that the bone marrow undergoes a compensatory hyperplasia as the result of the blood destruction. The histology of the marrow, however, does not tend to support this theory. The evidence of Zadek,⁶ together with that presented above, shows that the characteristic megaloblastic hyperplasia is most highly developed in severe relapses, and disappears, completely or in large part, during the remissions. This, in itself, might be interpreted as meaning that the hyperplasia recedes as soon as the hemolytic process ceases. It is, therefore, significant to observe further that the megaloblastic hyperplasia begins to decrease, and the cytology of the marrow becomes more normal, very early in the development of a clinical and hematologic remission, and at just the period when one might expect a compensatory hyperplasia to be most marked. In Case 1, just before a remission started, there was a slight increase of fat (indicating a less cellular marrow) with an increase of cells more mature than megaloblasts, and after a complete remission had taken place the marrow showed only slight signs of

biopsy, even when this was taken during a severe relapse. The most obvious explanation is that the phenomenon occurs only postmortem, but there are several points that cast doubt on such an hypothesis. Thus it must be remembered that while phagocytosis of red blood cells may be found in any type of marrow at necropsy, it is particularly constant and prominent in pernicious anemia. In addition to this, it has often been found in bone marrow 1 to 2 hours after death (the necropsy in Case 5 was performed 2 hours after death), and shows no tendency to be more marked if the autopsy is performed later. The question could be settled by the examination of tissue taken a few hours or days before death, but such material is not at hand. The phagocytic cells are clasmatocytes and, according to Sabin, they are derived from endothelial cells. In marrows obtained at biopsy, it is extremely hard to distinguish clasmatocytes from endothelial cells as they are obscured by the confused mass of myeloid cells (just as fat cells may be invisible in a very hyperplastic marrow), but when they have ingested erythrocytes and normoblasts, the clasmatocytes become enlarged and are easily seen. It is possible that clasmatocytes are actually increased in number from an early stage in the disease, but that they become phagocytic only in the terminal stage. If this is shown to be true it would be a fact of considerable importance, for it would indicate that the pathology of pernicious anemia is characterized by the proliferation of two derivatives of the endothelial cell, the megaloblast and the clasmatocyte, and it might be possible to go back one step further and consider whether the primary lesion is not associated with the endothelial cells. Rich¹¹ showed that clasmatocytes, grown *in vitro*, ingest red blood corpuscles with which they are brought in contact. Contact between clasmatocytes and erythrocytes seems to lead to phagocytosis. Is it possible that in the terminal stage of pernicious anemia the red blood corpuscles are not delivered to the circulation, but remain in the marrow, where they come in contact with large numbers of clasmatocytes by which they are ingested?

The fact that megaloblasts are not frequently found in the peripheral blood, even during a relapse when they are numerous in the bone marrow, and the comparative rarity of other immature forms in the blood stream, is best explained by Key's¹² observation that immature red blood cells tend to adhere to one another. Under such

the megaloblastic hyperplasia of pernicious anemia produces a bone marrow with diminished functional capacity, and it leads to the belief that this type of anemia is the result of the pathologic lesion in the bone marrow.

The histologic material now at hand, can only suggest why it is that the megaloblastic hyperplasia of pernicious anemia produces a bone marrow of diminished functional efficiency. At the height of a relapse, when the output of cells from the marrow is at its lowest, there is an extraordinarily rapid and extensive proliferation of megaloblasts, but the relative number of more mature cells in the bone marrow, normoblasts and erythrocytes, is usually diminished. During the progress of a remission on the other hand, when the marrow is hyperactive, there are fewer megaloblasts, but many more normoblasts and erythrocytes. The relapse is thus characterized by the rapid proliferation of primitive cells, and by a diminished tendency towards the differentiation of the more mature forms of the erythrocyte series, while the onset of a remission is marked by a resumption of a more normal process of cell differentiation. The cause of the anemia would thus appear to be an abnormal type of cell growth consisting in a development of the primitive megaloblasts, and a failure of differentiation of the more mature red blood cells that normally get into the peripheral blood. There is little to indicate whether this is to be regarded as a hyperplasia due to some extraneous toxin, or whether the process is similar to that of a tumor growth. The extraordinary clinical results that have been obtained recently in the production of remissions in pernicious anemia by the feeding of large amounts of liver¹⁰ suggest that this organ possesses some factor which affects cellular metabolism, and promotes the differentiation of the more mature cell types.

In addition to the above, it is worth noting that the venous sinuoids, into which mature erythrocytes are normally discharged, are extremely narrow and compressed in specimens of highly cellular marrow, and it is conceivable that a decrease of the vascular bed is a secondary factor in preventing red blood cells from getting out of the marrow.

It is also worthy of note that the phagocytosis of erythrocytes, which is such a striking feature in the bone marrow obtained at necropsy in almost all cases of pernicious anemia (see Case 5, bone marrow at necropsy), has rarely been observed in tissue obtained at

more normal type of cell development with an increased production of normoblasts and erythrocytes.

5. It is suggested that the striking clinical results obtained by the feeding of large amounts of liver in the production of prompt and marked remissions may be due to some factor in the liver which affects cell metabolism and promotes the development and differentiation of mature red blood cells.

I am greatly indebted to Dr. W. R. Castle for assistance in obtaining the pathologic material, to Miss E. Piotti for the drawings, and to Dr. Henry Jackson, Jr. for the photomicrographs.

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circumstances they would not be easily displaced from the marrow into the circulating blood.

If pernicious anemia be considered as primarily due to a bone marrow lesion rather than to a hemolytic process, the question naturally arises as to how one can explain the excess of bilirubin which is found in the blood plasma. In the absence of more definite knowledge of the physiology of pigment metabolism, one can only suggest that it results from an excess of pigment over and above what the marrow can use in constructing erythrocytes. This is consistent with the fact that bilirubin is increased during relapse, when the marrow is inefficient, and falls soon after the onset of a remission. It is also in harmony with the conception of Whipple¹³ who regards the disease as being due to the decreased formation of the stroma of red blood cells, rather than to the lack of the constituents of hemoglobin. The erythrocytes in pernicious anemia are, indeed, more than normally filled with hemoglobin. Reference may also be made again to that most definite type of hemolytic disease, congenital hemolytic jaundice, in which the amount of bilirubin in the plasma is many times greater than it is in pernicious anemia.

CONCLUSIONS

1. Observations on the structure of the bone marrow in pernicious anemia, made on tissue obtained at biopsy at different stages of the disease, show that the myeloid hyperplasia is most marked during relapse, and that the structure of the marrow tends to return to normal during remission.

2. During relapse the essential histologic lesion is a rapid and extensive proliferation of primitive cells (megaloblasts), with a relatively diminished tendency towards the differentiation of mature cells of the erythrocyte series. The bone marrow shows a cellular hyperplasia, but it is functionally inefficient.

3. Remissions are characterized by the presence of few megaloblasts and a great relative increase of normoblasts and mature red blood cells in the bone marrow.

4. The anemia of the relapse is explained by the functional ineffectiveness of the bone marrow, which results from the failure of the megaloblasts to differentiate towards mature erythrocytes. The blood picture of the remission is explained by the resumption of a

PLATE 61

- FIG. 10. Case 3. Jan. 18, 1926. To illustrate the extensive hyperplasia of megaloblasts and the presence of fat in the terminal relapse. $\times 500$.
- FIG. 11. Case 3. Sept. 2, 1925. General character of marrow as patient was going into a relapse. Note presence of fat containing fat cells, and also marked hyperplasia of megaloblasts. $\times 750$.
- FIG. 12. Case 5. Sept. 2, 1925. Clump of megaloblasts developing in inter-sinusoidal capillary between fat cells. Note mitosis. $\times 1000$.

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DESCRIPTION OF PLATES

PLATE 57

- FIG. 1. Case 1. May 19, 1925. General character of marrow during severe relapse. Note complete absence of fat. $\times 500$.
- FIG. 2. Case 1. Same as Fig. 1, but under higher power to illustrate extensive hyperplasia of megaloblasts, with mitoses, and relative scarcity of normoblasts. $\times 1000$.

PLATE 58

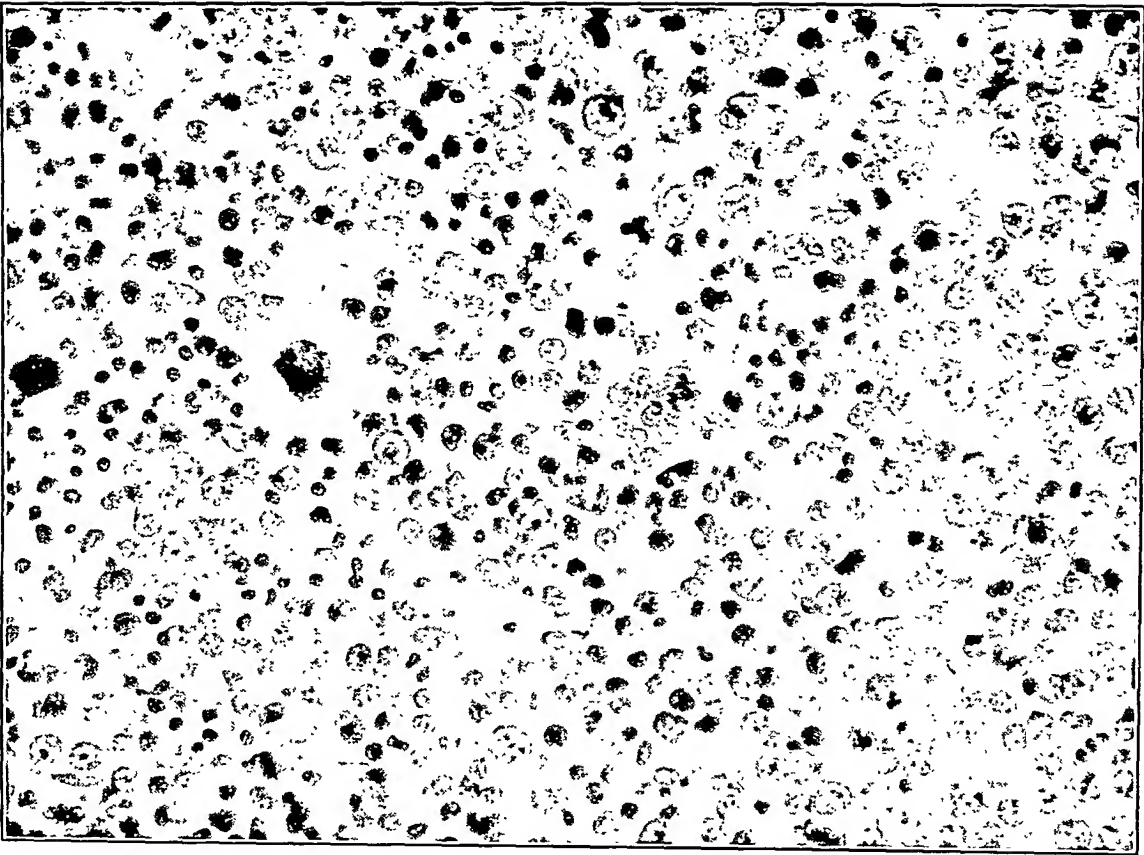
- FIG. 3. Case 1. May 19, 1925. Drawings of same material as Figs. 1 and 2. To illustrate hyperplasia of megaloblasts and numerous mitoses of megaloblasts. $\times 1250$ (approx.).
- FIG. 4. Case 1. Drawing similar to Fig. 3. $\times 1250$ (approx.).

PLATE 59

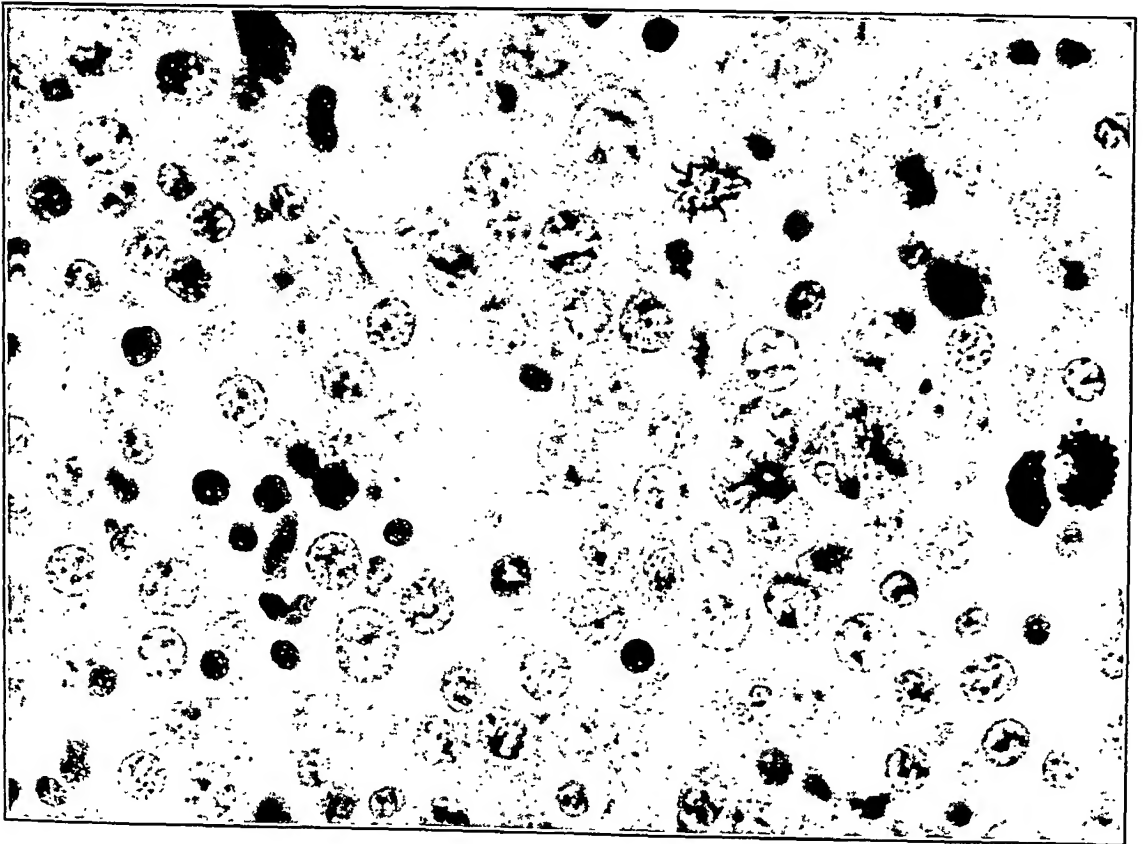
- FIG. 5. Case 1. March 12, 1926. General character of marrow during a remission. Note large deposits of fat and small islands of myeloid cells between the fat globules. $\times 100$.
- FIG. 6. Case 2. April 29, 1926. General character of marrow taken early in a remission. $\times 200$.
- FIG. 7. Case 1. March 12, 1926. Marrow during remission. Island of cells between fat globules to show predominance of normoblasts. Very few megaloblasts. $\times 750$.

PLATE 60

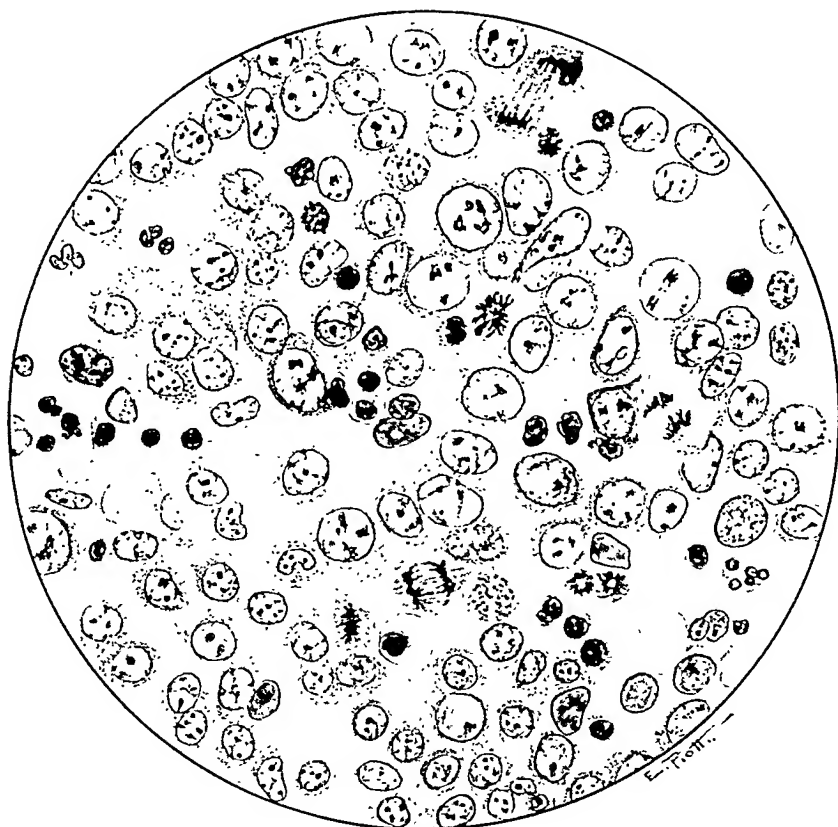
- FIG. 8. Case 2. March 12, 1926. General character of marrow during relapse. Hyperplasia of megaloblasts and displacement of fat. $\times 500$.
- FIG. 9. Case 2. April 29, 1926. Masses of normoblasts and erythrocytes in the intersinusoidal capillaries between fat globules during a remission. Very few megaloblasts present. $\times 1500$.



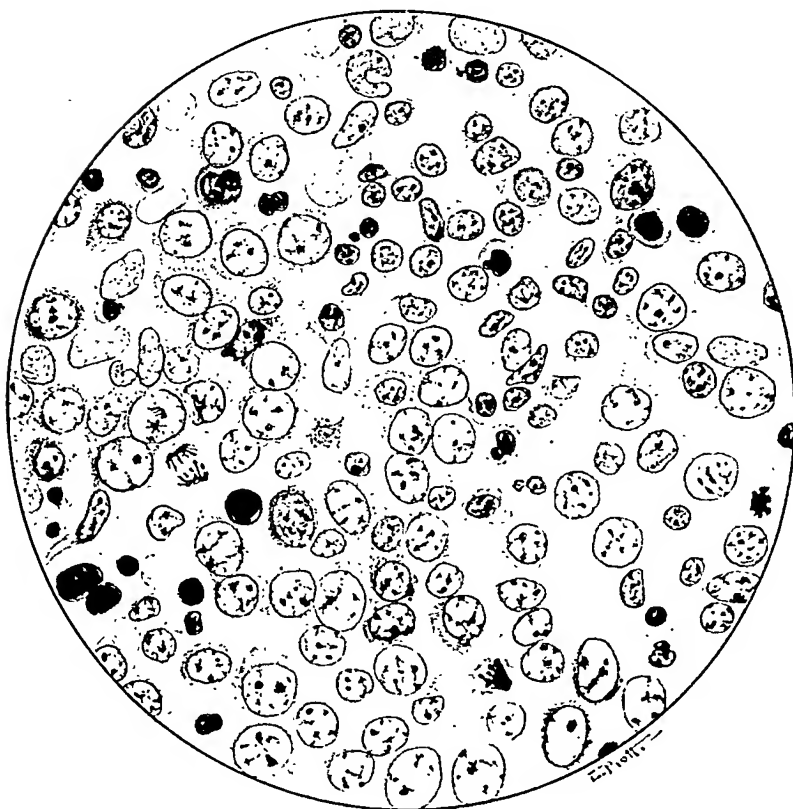
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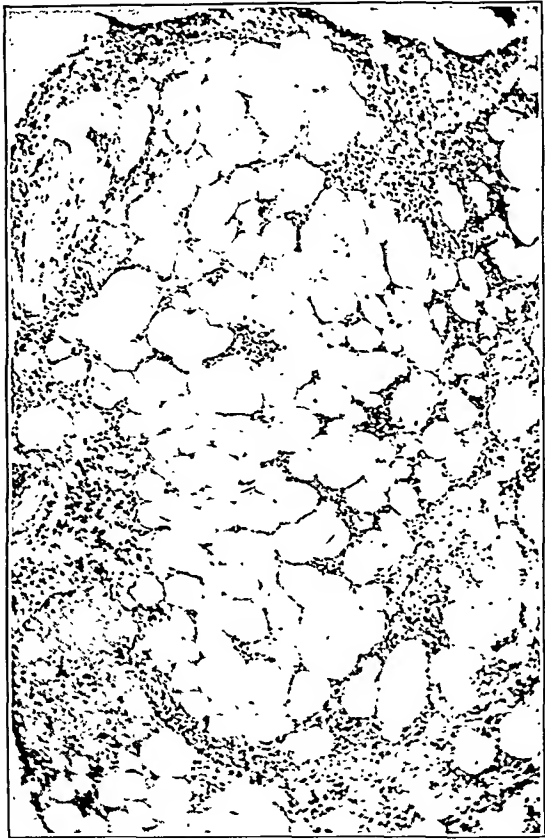
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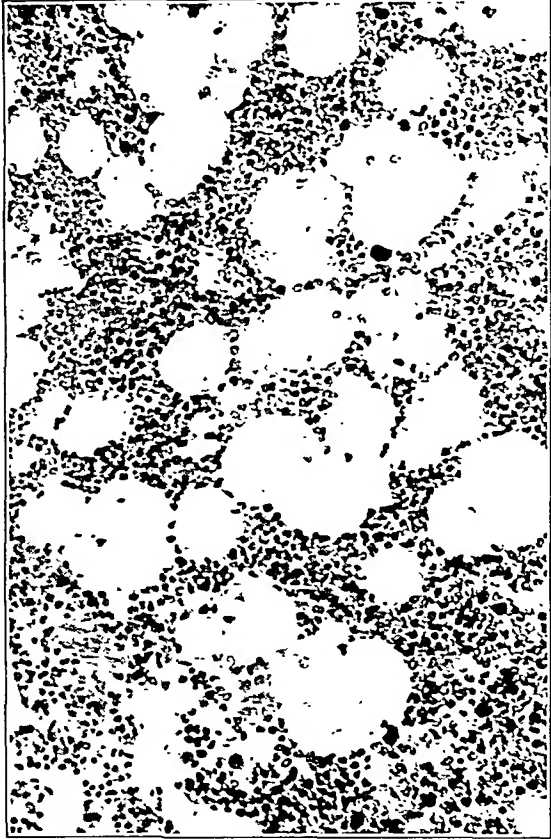
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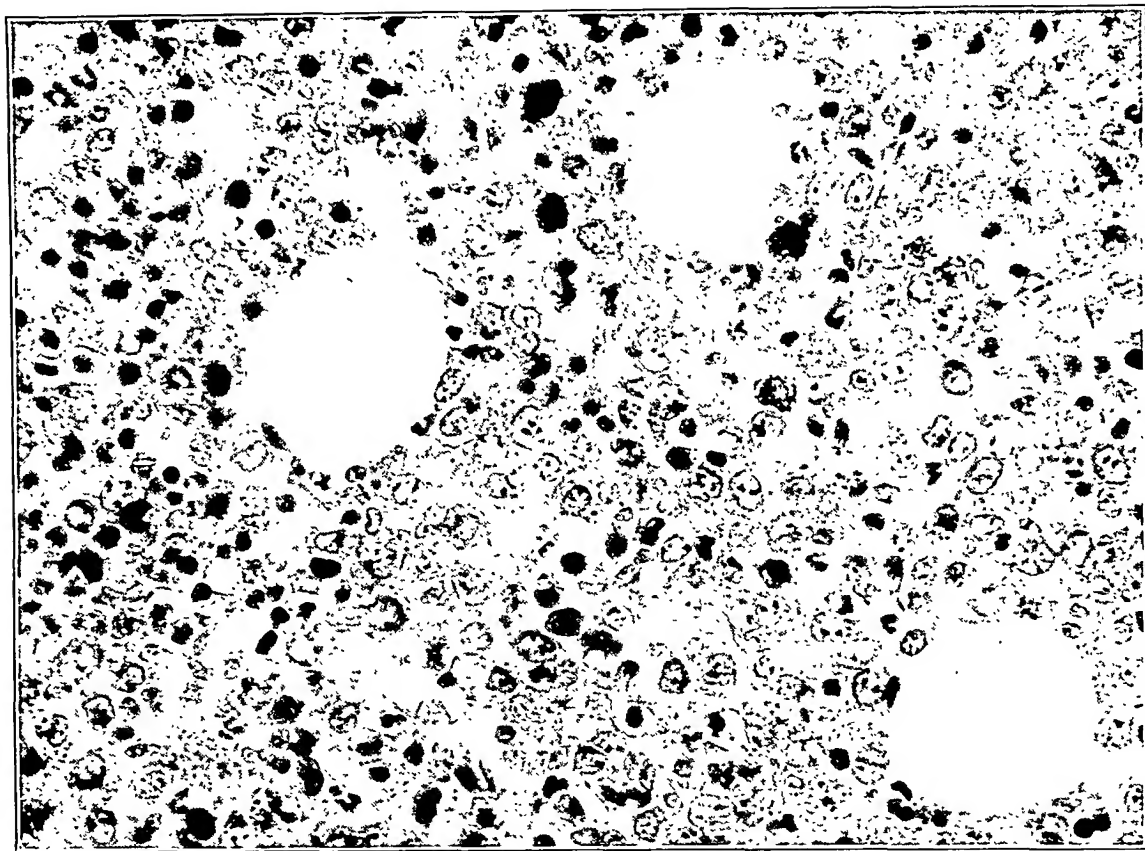
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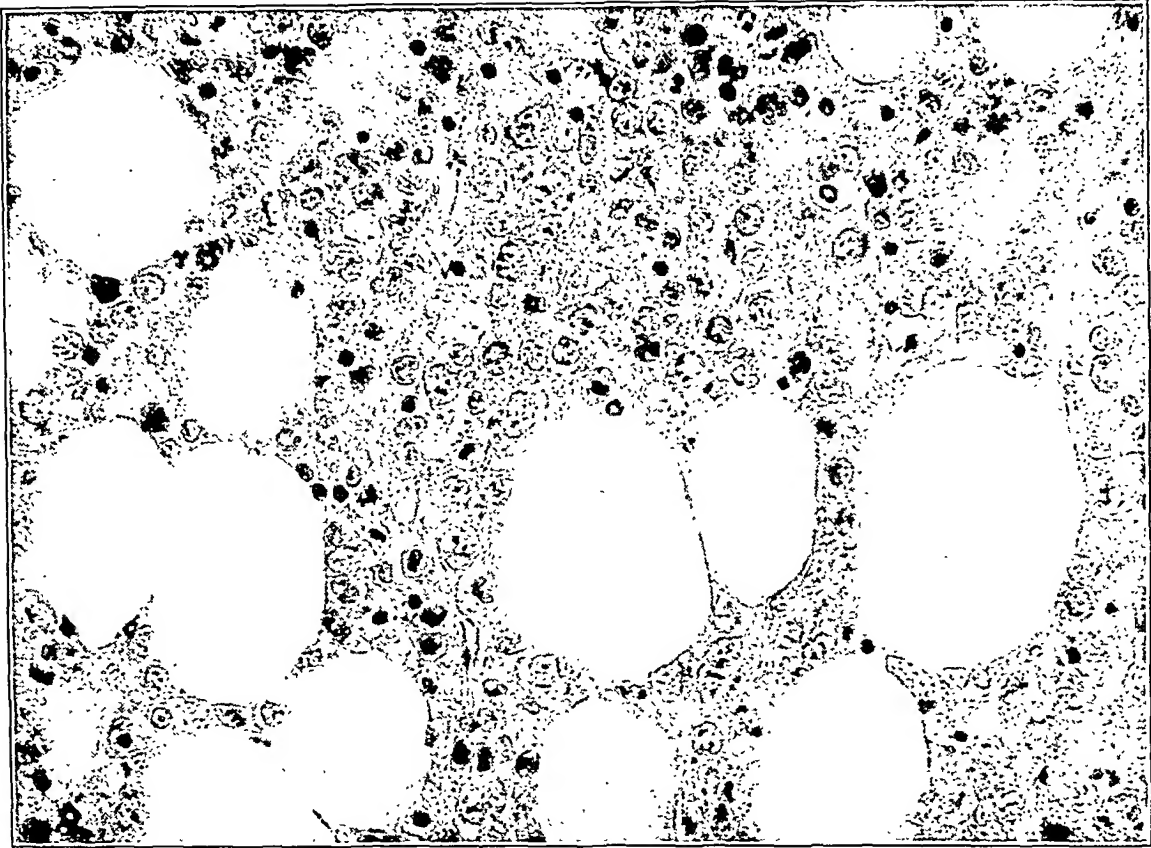
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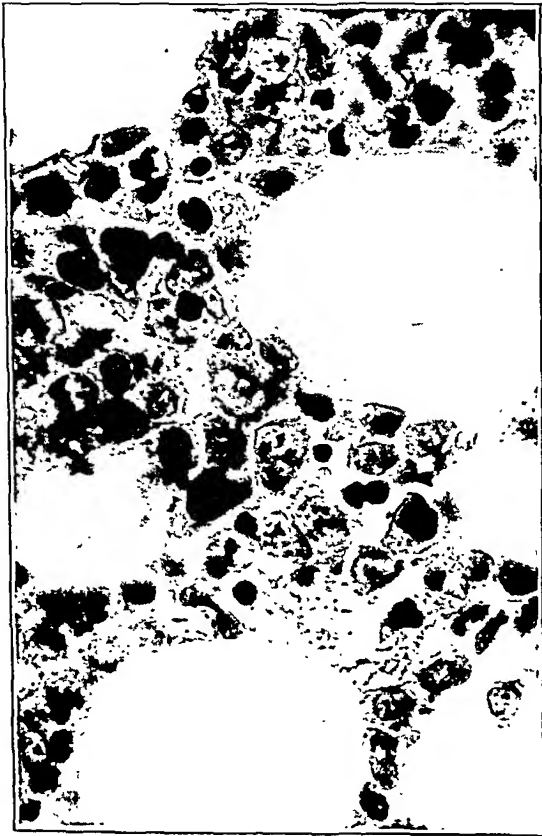
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11

Peabody



12

Bone Marrow in Pernicious Anemia

bellum of children and which are a common source of the so-called "sarcomatosis of the meninges," they preferred the name medulloblastoma.

In surprisingly few of the 400 tumors comprised in their study were true nervous elements identified, and in only three did they feel that the term neuroblastoma was justified. Among the true tumors of the central nervous system in the collection at the time of their report, no example of the still more highly differentiated type of growth known as a ganglioneuroma had been observed.* However in their monograph brief reference was made (pp. 93-94) to the case that we propose to report here in some detail. Though intraspinal, it was an extradural lesion and consequently belongs more properly in the category of peripheral than of central nervous system tumors. Both from the standpoint of its clinical history as well as on pathologic grounds, the case has most unusual features. It was included among the eighty examples of sarcoma which reacted favorably to bacterial toxins, as reported in 1913 by Dr. William B. Coley at the 3rd International Conference of Cancer Research.†

CASE REPORT

P. B. B. H. Surg. No. 14560. *A paravertebral swelling in the back of a paraplegic infant had proved on exploration (1911) to be a malignant tumor, "a spindle-celled sarcoma." Under Coley's toxin treatment the growth disappeared but the paraplegia persisted. A laminectomy 10 years later (1921) disclosed an intraspinal but extradural tumor which proved to be a "ganglioneuroma." Improvement of paraplegic symptoms during succeeding 5 years.*

May 18, 1921. Admission of William W., aged 11, with the complaint of spastic paraplegia. From the child's father, a physician, checked by Dr. Coley's published record of the case, the clinical history may be pieced together as follows.

The child was born on June 29, 1909. In February, 1911, the father was thrown from a wagon while holding the infant in his arms. It was not known at the time that the child had been in any way injured and the first suspicion of this was aroused a month later by a progressive weakness in the legs associated with a paravertebral swelling opposite the middle of the scapula on the right side. This swelling increased in size and the child was taken to be seen by a well known surgeon, Dr. Stuart McGuire of Richmond, Virginia.

The anamnesis records that the case was supposed to be one primarily of infantile paralysis to which the paravertebral swelling was unrelated. Gradu-

* A ganglioneuroma of the pineal body has since been encountered.

† The treatment of malignant inoperable tumors, with the mixed toxins of erysipelas and bacillus Prodigiosus. Case 38. Brussels, 1914.

Some of the inaccuracies in Dr. Coley's report, particularly in relation to dates and in regard to the situation of the lesion, which he states was above the clavicle, are herein corrected.

THE TRANSFORMATION OF A MALIGNANT PARAVERTEBRAL SYMPATHICOBLASTOMA INTO A BENIGN GANGLIONEUROMA*

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INTRODUCTION

Among the rarest of the tumors of the central nervous system are those composed of the highly specialized nervous elements themselves. There are some pathologists who even doubt the existence of growths in which these elements chiefly participate, though, as is well known, occasional fully developed nerve cells may be disclosed in some of the gliomas when examined by the more modern methods of differential staining.

Certain tumors which take their origin from the sympathetic nervous system and more particularly in association with adrenal rests were described by J. H. Wright¹ as neuroblastomas; and subsequently tumors with a similar cellular architecture found in the central nervous system came to be thus designated. Wolbach and Bailey,² for example, in a brief article on the subject employed this term, as others had done, for certain tumors, with stroma, composed of round cells having delicate cytoplasmic processes, many examples of which had come under observation in the clinic.

Bailey and Cushing,³ however, in a comprehensive reinvestigation of the tumors of the central nervous system occurring in the Peter Bent Brigham Hospital series found exceedingly few examples of what they felt justified in calling neuroblastomas. They consequently were disposed to regard this term as previously employed for the J. H. Wright type of tumor, at least in so far as it occurs in the brain, as a misnomer. In their opinion, these tumors arise from primitive and undifferentiated elements ("medulloblasts") of the nervous system. Potentially the cells therefore may be capable of differentiating into either glial or nervous elements, but, as a matter of fact, their disposition is toward a transformation into glia. Accordingly, for these tumors which are frequently encountered in the midcere-

* Received for publication December 24, 1926.

On examination the child shows marked cerebellar attitude of the head, though the attitude referable to one lobe is not constant. Frequently there is a marked tremor of the head, precisely of the same character as that seen in advanced cases of disseminated sclerosis of the cerebellar or medullary type, or of advanced cases of Friedreich's ataxia. The pupils are brisk, the light and accommodation equal, central and regular in outline. There is no ptosis. There is marked nystagmus on lateral conjugate movement, either to the right or to the left. Query, weakness of the left sixth nerve. Jaw deviates always to the right, therefore, query, right motor fifth nerve affected. The masseter on the right side contracts less well than that on left. Child apparently hears on both sides. There is obviously no defective vision, no changes in the sensibility of the face, nor is there any facial palsy. The tongue comes out straight, and there is no tremor, or wasting. Palate normal.

Upper extremities. There is marked motor ataxia in both arms of definitely cerebellar type. (*N. B.* This ataxia is not in any way dependent on any sensory defect.) Diadokokinesia right and left. The arms are very strong for all movements. There is no wasting. No paralysis in the legs. All movements can be performed, but the ataxia manifested in the arms is present in the lower limbs also. The sensory condition is everywhere normal, that is to say, the child appreciates pin-prick, touch and temperature everywhere. *There is no segment of anesthesia or hypesthesia in any way corresponding to the situation of the tumor in the mid-dorsal region.*

Reflexes. Arm jerks normal. Abdominal reflexes right and left, present and equal. Knee jerks increased equally. Double extensor responses. Owing to inattention of the child combined with gross nystagmoid movement of the eyeballs, it was difficult to get a clear view of the optic discs. The veins in each ocular fundus were very large. Outer edges of discs were seen in glimpses and were apparently clear, and did not present the sinuosity of outline nor the blurring usually seen after the subsidence of a neuritic process.

We are told that the child has improved very greatly in the past six months not only as regards weight and general nutrition, but in regard to power and ability to perform movements. It is obvious, however, that there is still a gross cerebellar lesion.

These notes made by a highly competent observer surely indicate that the cerebellar symptoms at the time were predominant and that the spinal paraplegia had largely disappeared. Did the story end here, one might well enough assume that the symptoms had been produced, as Dr. Ewing's qualifying remark suggested, by one of the common tumors (medulloblastomas) of the fourth ventricle which had inoculated the cerebrospinal spaces and caused a spinal implantation with paraplegia, which in certain rare cases is known to disappear spontaneously.

It may be pointed out, however, that the child was then under 3 years of age (2 years, 9 months, to be exact); also that the reflex movements of the lower extremities in transverse lesions of the cord were at the time imperfectly understood, and even today when

ally the weakness progressed up the trunk and by May 1, 1911, the arms could not be moved; and soon there was fever, nausea, vomiting and stupor. There was also loss of sphincteric control. The eyes became crossed and there was ptosis.

In view of these symptoms Dr. H. Allison Hodges, a neurologist, saw the child in consultation with Dr. McGuire and the opinion was then expressed that the syndrome was due to some intradural extension of a disease which was possibly tuberculosis, though a lumbar puncture was negative and there was neither stiffness of the neck nor Kernig's sign. Since the paravertebral swelling was increasing in size, it was decided at the father's request that an exploratory incision be made to determine the nature of this local lesion.

This preliminary story is important, if it can be relied upon, in showing that there was originally a widespread involvement of the central nervous system, which extended above the level of the swelling in the back. To continue:

The paravertebral tumor was by this time plainly palpable and semifluctuant. It suggested either a lipoma or a tuberculous abscess. Sometime in June, 1911, Dr. McGuire, by an incision parallel to the ribs (Fig. 1), exposed the growth and found a well defined tumor apparently springing from the lamina or right transverse process of the sixth thoracic vertebra. The lesion was thought to be inoperable, and after removing a piece of tissue for histologic examination, the wound was closed. This tissue was diagnosed fibrosarcoma and the prognosis seemed hopeless.

The surgical incision healed promptly but since the tumor continued to increase in size, treatment by toxins was begun (*circa* July 1, 1911) by the child's father under Dr. Coley's direction.

Apparently it was not until the next year, in March, 1912, that the case was first seen by Dr. Coley. At that time Dr. James Ewing, after an examination of the original slides, stated that the growth was unquestionably "a malignant tumor which might very well be called a sarcoma." But this he qualified by the further remark: "I am inclined to think it is either an endothelioma secondary to the cerebral growth, or possibly a neurocytoma derived from misplaced nerve tissue in the cranium."

This statement is quoted to show that the view still prevailed that there had actually been an intracranial process and that the local lesion in the back was not the sole cause of the clinical picture. A detailed neurologic examination of the child made on March 19, 1912, by Dr. Foster Kennedy would appear to settle any doubts that might prevail on this score at the present day. He is quoted as follows:

had some voluntary control. He could stand alone with the support of crutches and by taking advantage of a sustained adductor spasm which held the knees together, the feet being separated and turned in. When this spasm relaxed he would fall unless supported.

Sensation was apparently completely lost up to the level of the sixth thoracic skin-field, but the sensory tests were difficult to interpret because a pin-prick or even a light touch would evoke spontaneous reflex movements which gave sensory impressions referred by the child to his legs.

The deep reflexes both at knee and ankle showed an easily elicited and sustained clonus. There was on both sides an active dorsal toe response to almost any form of stimulation, even such as the mere exposure of the legs by removing the bed covers. The cremasteric reflexes were active. Reflex erections were easily provoked by pricking the glans or picking up the skin of the groin. Under these circumstances the legs would flex and the bladder which could retain about 200 cc., would be emptied without sensation (Figs. 2 and 3).

Whatever may have been the condition ten years previously, certainly at this time there was nothing to be seen but the evidence of a total transverse spinal lesion at about the level of the sixth thoracic segment which corresponded to the site of the original paravertebral tumor. Moreover, the X-ray plates disclosed a somewhat dense shadow in the region of the former tumor which however did not affect the normal outlines of the adjacent laminae or transverse processes. It would appear from the hospital history that no pre-operative diagnosis was ventured. No lumbar puncture was performed. It would almost certainly have shown a complete block with xanthochromia.

May 21, 1921. *Operation (Cushing). Laminectomy with disclosure of sharply defined extradural mass of dense non-infiltrating and non-adherent tumor tissue. This mass encircled and constricted the meninges and cord and apparently communicated with the relic of the original lesion through an enlarged intervertebral foramen.*

With the position of the old cicatrix as an indication of the original tumor site, the spines and laminae of the three adjacent vertebrae were at first removed. There was no apparent lesion of the bones but, on scraping off the periosteum from the laminae of the right side, a dense scar-like tissue was encountered in the spinal muscles which was taken to be the residuum of the original lesion. On the removal of the spines and laminae, instead of the usual extradural cuffs of fatty tissue, the canal was found to be filled with the same kind of dense scar-like tissue. An incision, made into this firm tissue, was carried down to a considerable depth without disclosing dura. The tissue was quite vascular.

Realizing that the exposure was insufficient, the additional spines and laminae of the two preaxial vertebrae were then removed. This brought into view the upper margin of the lesion with normal-appearing dura headward to it. Similarly the laminectomy of an additional postaxial vertebra exposed the normal dura caudad to the lesion. It therefore extended over approximately five spinal segments.

observed in infants may be difficult to distinguish from spontaneous movements. It would appear, nevertheless, that the intracranial and cerebellar symptoms were more pronounced at the time than were those referable to the spine.

The child's father continued the injection of the toxins, and eight months later (Nov. 23, 1912) wrote to Dr. Coley as follows: "There is no indication of the return of the growth on the back. He has never regained the use of his legs though he can move them better and they show no signs of wasting or contractions. His eye symptoms are also better. He is hearty and well developed, does not seem to suffer any, and is bright and full of life." And after another eight months (July 25, 1913): "Am glad to say that my little boy's condition is somewhat improved. He can use his legs but little; he can move them but has no strength in his knees. There is no sign of a return of the growth on his back, and his general health is good; his mind seems bright. I give him four minims of the toxins about every third day." During this time apparently the cerebellar symptoms, if such they were, seem to have fallen into the background of the picture.

For the ensuing eight years, the child, though remaining paraplegic, thrived and developed in all other respects. He had acquired an automatic control of the bladder and rectum and by 1919 had learned to balance himself awkwardly on crutches. As the spinal condition seemed stationary the father had finally come to the conclusion that an exploratory laminectomy should be undertaken. Hence the child was admitted to the Peter Bent Brigham Hospital under the date specified, just ten years after his original operation. The child was well nourished, healthy, coöperative, and had *no discomforts whatsoever*.

May 18, 1921. *Neurologic Examination*. This showed absolutely no signs of involvement of the brain or upper spinal cord. There was not a trace of the nystagmus, diplopia, ptosis and so on, described in the previous history. In the back was the scar of the old operation (Fig. 1). This was soft and movable, and palpation revealed no evidence of an underlying tumor. The X-rays of the spine, however, showed a cloudy area representing either dense fibrous tissue or ossification at the site of the original lesion. The outlines of the laminae, spines and transverse processes were clear and without evidence of having been involved in the disease.

The child was powerless to move the lower extremities but the slightest stimulus served to throw them into reflex movements which strongly suggested voluntary movements and even the patient was under the impression that he

second piece of tissue from the extravertebral region consists of translucent white fibrous tissue.

From the first frozen sections at the time of the operation, the impression was obtained that it was degenerated dorsal root ganglion surrounded by cicatricial tissue, but on sectioning the larger piece, the material was found to consist of fat tissue traversed by bands of fibrous and nerve tissue. There were nerve bundles running in all directions, and masses of nerve tissue containing ganglion cells, many of which were much degenerated though easily recognizable because they were surrounded by capsular cells. Before the close of the operation a small piece of muscle from the erector spinae group, opposite the level of the tumor, and a few additional pieces of gray fibrous tissue possibly fascia were excised and submitted for study.

Microscopic Report. Many blocks were made of the tissue received. They were stained with eosin-methylene blue, Van Gieson's stain, and phosphotungstic acid hematoxylin. The tissue unfortunately had all been fixed in Zenker's fluid so that impregnation by Cajal's method was precluded. The tumor has invaded fat and voluntary muscle and consists mainly of fibrous tissue with islands of large cells having one to several nuclei. The fibrous tissue cells are characterized by abundant, wavy, intercellular material which stains pale brown with phosphotungstic acid hematoxylin and pink with Van Gieson, and is identical in appearance and staining reactions with the intercellular material of peripheral neurofibromas and tumors of the acoustic nerve (Fig. 4). With the phosphotungstic acid stain, one can trace long, loose, deeply staining processes which appear to be axis-cylinder processes, and here and there in the fibrous tissue there is a suggestion of myelin sheaths.

The large cells have many different shapes and sizes. Many of them resemble mature ganglion cells which send off dendrite-like processes for long distances. Others unquestionably have axone processes (Fig. 5), which can be followed for long distances and show minute granulations arranged linearly. The presence of Nissl substance in these cells is strikingly demonstrated by the Bielschowsky-Plein method (Fig. 6). A few of these cells presenting all the appearances of ganglion cells are surrounded by capsular cells.

In addition to the large ganglion cells, there are cells scattered in groups which exhibit the same intense basic staining and suggest the possibility of being embryonic elements in the tissue (Fig. 7). There

The exposed growth was then tilted up and lifted by blunt dissection away from the dura to which it was not adherent. As the fairly rigid posterior shell of tumor was broken away from its lateral attachments, it was evident that it extended around to the anterior aspect of the canal on the right side where it became much thinned out.

A fragment of this intraspinal tissue was immediately examined (Wolbach) and was reported as a probable ganglioneuroma. With this suggestion of a possible seat of origin for the tumor in a posterior root ganglion, which might account for its hourglass shape by coincidental extension into the paravertebral muscles and spinal canal, the wound, before closure, was reinvestigated. The extravertebral mass which had been taken to be cicatricial tissue was found to lie mainly between the laminae of the fifth and sixth thoracic segments just opposite the thickest portion of the residuum of the intraspinal growth. The two masses appeared to communicate by a narrow neck through the region of an enlarged intervertebral foramen.* A fragment of the dense extravertebral tissue was then removed for comparison with that which had been removed from within the canal.

Following the removal of the tumor the greatly compressed dura filled out to its normal dimensions and resumed its pulsations. It was not opened. In the hope that the procedure would suffice to release the cord from its constriction and permit a return of function, if such a thing were possible after so many years of compression. The wound was closed as customary in successive layers.

Postoperative Report. The child made an excellent recovery from the operation. Healing was perfect (Fig. 1). The preoperative symptoms remained unchanged at the time of his discharge.

Subsequent Notes. The boy's father soon wrote that he had improved greatly and had much better sphincteric control. Again, in 1923, owing to a persistent adductor spasm, some tenotomies were performed which helped him greatly. Now (August, 1926), five years since the laminectomy, he writes to say that conditions have continued to improve; that "the boy rarely has to void at night, and has not wet his bed for over two years"; and that he does not have to void frequently by day and can hold his urine for about fifteen minutes even after there is inclination to void. He reports that the cutaneous sensation seems "to be good," and states that "he gets up, dresses himself, and walks around the room without a crutch though most of the time he uses one crutch; he hitches his pony himself and drives around anywhere he pleases."

THE HISTOLOGY OF THE LESION (Wolbach)

Gross Description. The intraspinal specimen consists of two fragments of fibrous tissue, one about 4 cm. long, the other slightly less, and about 2 gm. in total weight. Both fragments contain on palpation small, sharp, discrete nodules 1 to 2 mm. in diameter. The

* These hourglass-shaped tumors are well known. They are usually, however, of the type of the Recklinghausen tumor (neurinoma of Verocay). For the most part they are histologically unmistakable and have no relation to the tumor under discussion other than that they may have their histogenetic origin likewise in the cells of the neural crest. Cf., a recent article on the subject by M. Borchardt (*Klin. Wchnschr.*, 1926, v, 636).

studied at that time by a number of pathologists, and the presence of a more highly differentiated type of cell would not have failed to excite comment. The tumor disappeared or diminished (cicatized) under the influence of Coley's toxins. Symptoms of spinal pressure persisted, and years later (1921) the intraspinal lesion was identified as a ganglioneuroma, the more compact extraspinal remnant of the tumor showing the same characteristics.

A very thorough examination of the tissue removed in 1921 reveals no trace of cells similar in structure to those which in 1911 apparently constituted the entire growth. Instead we find a neoplasm composed chiefly of two elements, one representing ganglion cells and the other representing the growth of capsular and sheath of Schwann cells, which is the usual combination recorded in ganglioneuromas of sympathetic origin. Therefore, one is forced to conclude that the cells of this tumor as a whole have responded to the influences or factors governing the normal differentiation of the nervous system.

The change in the structure of this tumor during the ten-year interval may be regarded as throwing light on the potentialities of the cells in the so-called "sympathetic neuroblastomas," and supports the idea expressed by Bailey and Cushing that these lesions represent a tumor of a more primitive type of cell (sympathicoblast) than the sympathetic neuroblast. Whether the early story of a widespread process which involved the intracranial chamber with the production of cerebellar symptoms has any bearing on this localized tumor must remain purely conjectural.

It would appear without question that the proliferative activity of the normal growth subsided coincidentally with the administration of the bacterial toxins. It is safe to presume, from the study of the original tumor and its comparison with the tumor after a ten-year interval, that the lesion was originally an actively growing sympathicoblastoma whose cells, coincident with loss of proliferative activity, came to be differentiated in time into ganglion cells and into sheath and capsular cells. Whether or not this is the correct interpretation of the process, the case, from the pathologic standpoint, is a unique one.

are no mitotic figures and there is no suggestion of activity on the part of fibrous tissue.

In stating that the adjacent spinal muscles and fat have been invaded by the growth, I wish to indicate that both of these tissues have been incorporated in the rather compact fibrillary (neoplastic) tissue, and that groups of small and large ganglion cells are found in such regions. The ganglion cells for the most part resemble those of sensory ganglia, but occasionally there are large fusiform cells with two processes, one at each end. In addition, here and there in the tissue are circumscribed collections of lymphoid cells surrounding small capillaries; they resemble lymph follicle formation rather than an inflammatory reaction (Fig. 8).

The Original Tumor. Through the kindness of Dr. Coley, a section of the original tumor from which the diagnosis of sarcoma had been made was forwarded for our inspection. At first glance the resemblance of the tumor to a fibrosarcoma (Fig. 9) with very little intercellular substance might be conceded. However, a peculiarity is immediately noticed in that the tissue is partitioned by connective tissue bands having all the relationships of a stroma in an epithelial tumor. With the stain employed (hematoxylin and eosin), it is impossible to see cell outlines, and the impression of spindle-shaped cells is obtained wholly through the oval outlines of the nuclei. The nature of the tumor, however, is made apparent by the presence of an intercellular substance consisting of extremely delicate fibrils which are stained a faint bluish pink. These fibrils occur in bands of considerable width separating cell masses from the stroma, and as large bands joining widely separated groups of cells. The size and grouping of the cells, the connective tissue stroma and the presence of the delicate fibrils which resemble cytoplasmic processes of the tumor cells, agree perfectly with the characteristics of the so-called "sympathetic neuroblastoma." In the slide submitted there are no cells of a more mature type such as have been frequently described in neuroblastomas arising in the adrenal gland.

DISCUSSION

The importance of this long record lies largely in the interpretation of the pathologic changes which occurred in a tumor during a ten-year interval. Briefly, we have for consideration an intervertebral tumor of the hourglass type which lay partly within and partly without the spinal canal, but presumably was always extradural. The extravertebral expansion of the growth was explored in 1911 and was regarded as a spindle cell sarcoma. Subsequent study of a slide sent to us by Dr. Coley showed a structure typical of the sympathicoblastomas. Presumably this slide is representative of the tumor as a whole as it existed in 1911. The lesion was carefully

years old who had been operated upon for a supposed malignant tumor of the thyroid and who promptly succumbed. At necropsy it was found that the growth had arisen at the angle between the first and second thoracic vertebrae and the adjacent rib. The growth was a typical sympathicoblastoma with neurofibromatous areas. In a separate nodule of the tumor which had a perfectly benign appearance fully developed ganglion cells were present.

A similar case was reported in the same year by Freund.¹⁴ The patient was a child $4\frac{1}{2}$ years old. The tumor was on the right side of the neck apparently at the level of the thyroid, and lay between the carotid artery and internal jugular vein. It was removed surgically. The tenth nerve was not seen at the operation. Histologically there were well differentiated ganglion cells as well as groups of less differentiated cells resembling those of sympathicoblastomas, while the bulk of the tumor had essentially the structure of a "neurofibroma," accompanied by axis-cylinder processes and some myelin sheath formation.

Another case in a somewhat unusual situation was described in Dunn's second paper.¹⁵ The tumor which was surgically removed took its origin from deep in the sphenomaxillary fossa. The tissue proved to be moderately rich in the embryonic elements, which have wholly disappeared in our own case, but ganglion cells, sheath cells and axis-cylinders were typically represented. This, as Dunn states, represents an unfavorable omen as regards prognosis owing to the high potentiality for proliferation and metastasis possessed by these particular elements.

The unique feature of our present case report is the fact that an interval of a decade passed between the two occasions when the lesion was histologically examined. In all other recorded examples the tissue has been subjected to study at only one stage of development, or at least with but a short interval between a surgical biopsy and a postmortem examination.

SUMMARY

A paravertebral swelling at the sixth thoracic level occurred in the back of a child 2 years of age, following a trauma. The lesion proved on exploration to be a cellular sympathicoblastoma (sympathetic neuroblastoma) which was mistaken at the time for a sarcoma. The

LITERATURE

Comparatively few ganglioneuromas have been recorded. John Shaw Dunn ⁴ in 1915 collected fifty examples from the literature. The great majority of them (forty-one cases) were situated, as might be expected, in some part of the sympathetic nervous system. There were four cases in its cervical portion, five in the thoracic and twenty-nine in the abdominal chain or its branches, eleven having been in the region of the adrenal gland.

Their rarity among intracranial tumors is shown by the fact that out of more than 1000 histologically verified cases in the Peter Bent Brigham clinic, only one tumor that might be called a ganglioneuroma has been encountered and that arose from the pineal body. When they do occur in the intracranial chamber they usually take their origin from the cranial nerves. Those which actually arise from the central nervous tissues themselves are with difficulty distinguished from heterotopias. Achucarro ⁵ and Lhermitte ⁶ have each described such a tumor in the cerebellum, Pick and Bielschowsky ⁷ one in the medulla oblongata, Schmincke ⁸ and Dumas ⁹ in the cerebrum, and both Greenfield ¹⁰ and Robertson ¹¹ have encountered an example in the region of the tuber cinereum, probably arising from misplaced cells of the ganglionic crest. Bielschowsky ¹² has more recently described a case in which there were a number of small tumors in the ventricular walls which did not break through the subependymal glia; the author describes them as multiple ependymal ganglioneuromas.

As is well known, the sympathicoblastomas have often been described as consisting of two types of tissue. Masses of cells with scant cytoplasm and richly chromatinized nuclei, often arranged in rosette forms, are separated by connective tissue-like bands which with specific stains give the reaction neither of collagen nor of glia. They are for the most part highly malignant lesions and produce metastases, the undifferentiated cellular elements being those which metastasize.

Certain examples from the literature may be cited in evidence of the fact that these tumors originate from cells of a more primitive type than the neuroblast, and show a tendency in places to differentiate into fully formed nerve cells as well as into capsular or neurilemma cells. K. Martius ¹³ (1913) reported the case of a child 2½

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DESCRIPTION OF PLATES

PLATE 62

- FIG. 1. Showing the oblique white scar of the original operation (1911) and the recent scar of the laminectomy (1921).
- FIGS. 2 AND 3. Showing (above) patient in May 1921 with spastic rigidity of the lower extremities; (below) effect of pinching a fold of skin in the groin. Note involuntary flexor contraction with elevation of right heel from table; also slight erection with automatic evacuation of bladder, the typical lower spinal mass reflex.

PLATE 63

- FIG. 4. From a portion of the tumor having the general structure of a "neurofibroma" and containing a bundle of incompletely myelinated nerve fibers. Phosphotungstic acid hematoxylin stain. $\times 500$.
- FIG. 5. From a portion of the tumor containing ganglion cells in some instances accompanied by capsular cells. Phosphotungstic acid hematoxylin stain. $\times 500$.

PLATE 64

- FIG. 6. Cells selected to show Nissl bodies. Bielschowsky-Plein stain. $\times 850$.
- FIG. 7. From a portion of the tumor containing nests of ganglion cells in various stages of differentiation, surrounded by fibrous tissue such as is found in "neurofibromata," and which represents tumor elements of neurilemma or sheath cell origin. The small cells with round nuclei and deeply stained cytoplasm are immature ganglion cells and probably represent multiplying elements. Phosphotungstic acid hematoxylin stain. $\times 500$.

PLATE 65

- FIG. 8. Section to show collections of lymphoid cells which might easily be mistaken for persisting embryonal elements of the tumor. Perdrau's method. $\times 300$.
- FIG. 9. Drawing made from a section of the tumor removed in 1911. A field showing the nondescript appearance of the cells and the masses of delicate fibrils characteristic of a sympatheticoblastoma. Hematoxylin and eosin stain. $\times 500$.

tumor had apparently taken its origin from the region of an intervertebral foramen, and had extended into the spinal canal as well as into the spinal muscles: Apparently under the influence of Coley's toxins the activity of the growth subsided. Ten years later, owing to the persistence of a paraplegia, an exploratory laminectomy was performed. This disclosed a relic of the former growth whose cells had become completely differentiated into ganglion, capsular and neurilemma cells.

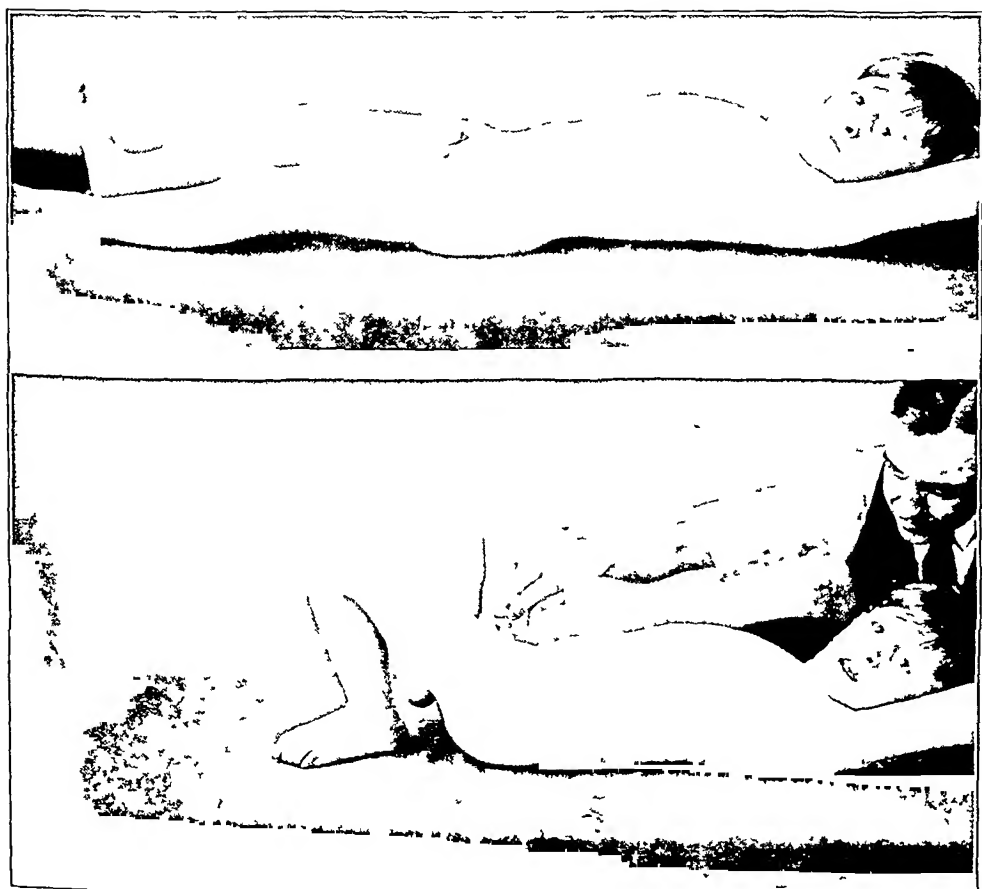
Because of the unusual circumstances which permitted a study of the lesion at two remote periods, the case illustrates particularly well what has been pointed out by others, that a sympathetic neuroblastoma may be the precursor of a ganglioneuroma.

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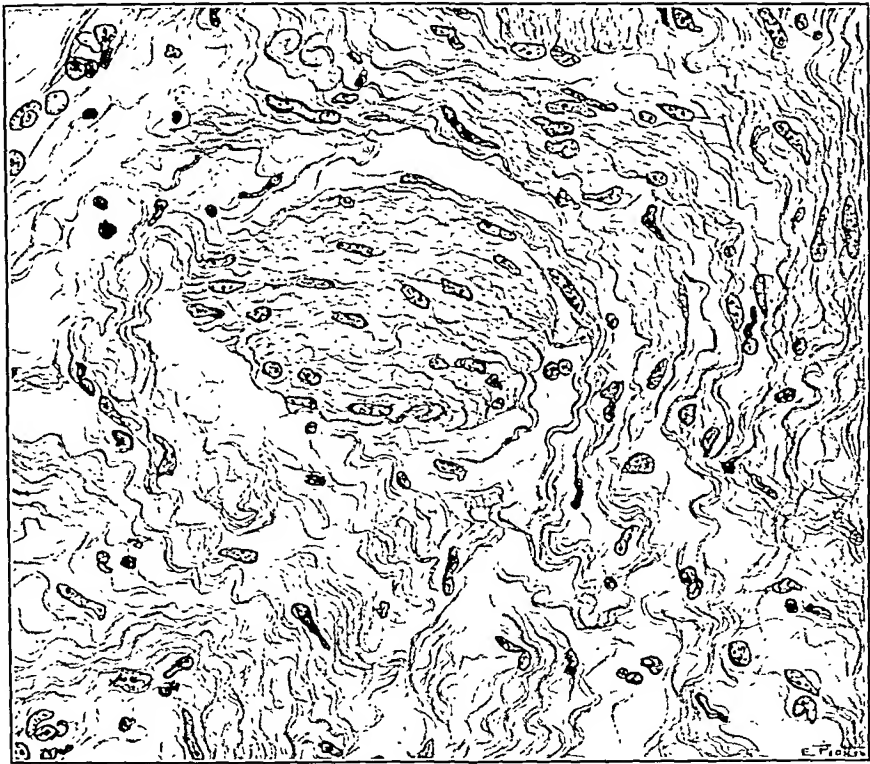
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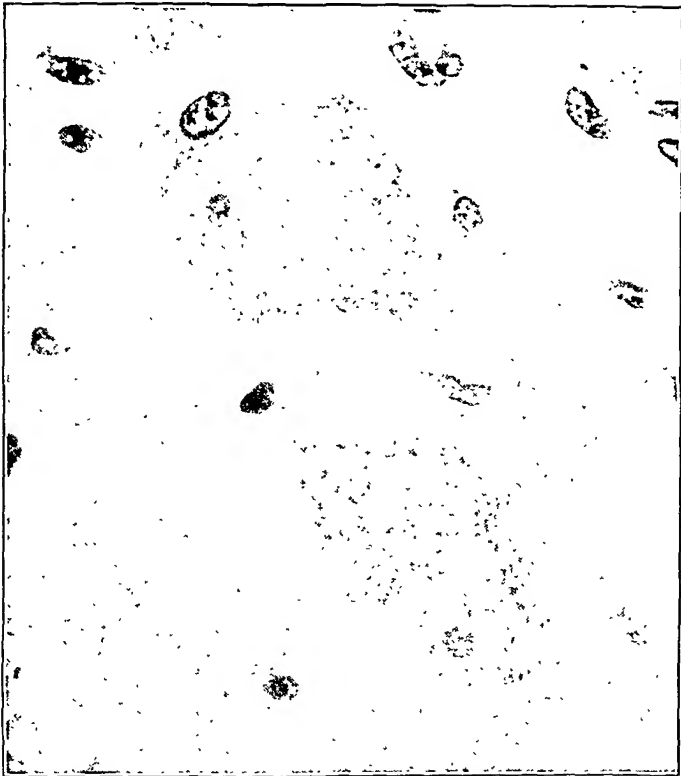
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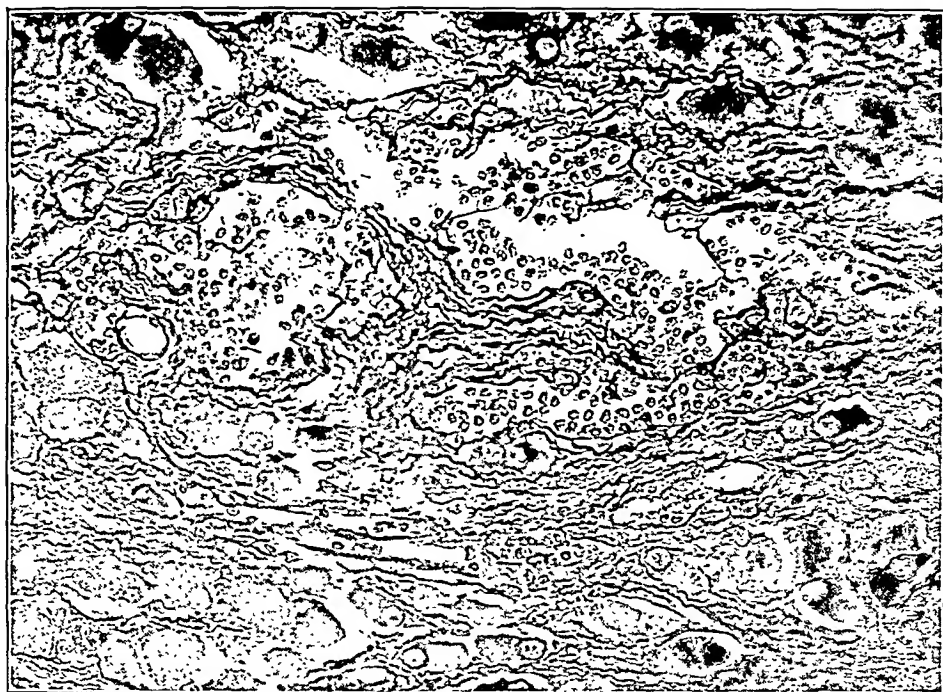
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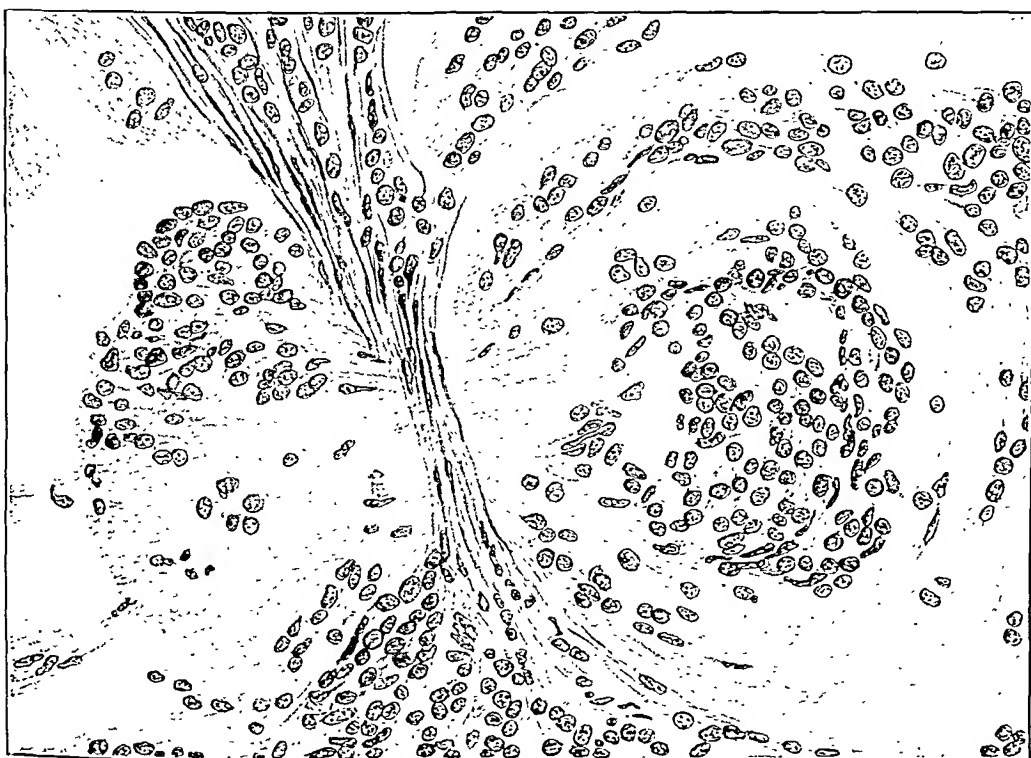
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Since none of the human material at my command showed the early stages in the development of a tubercle it was necessary to make use of experimental material. Accordingly a series of rabbits were injected through the marginal vein of the ear with 0.1 mg. of a bovine type of tubercle bacillus. The animals were killed 7, 14, 28 and 56 days after the inoculation. The lungs were fixed in 10 per cent formalin and impregnated by Ferguson's¹ modification of Bielschowsky's silver method.

No distinct tubercles were found in the seven-day rabbit but small collections of mononuclear leucocytes were present. The fourteen-day rabbit showed well marked mononuclear or "epithelioid" tubercles in an early stage. These contained in their center a fine network of reticulum (Fig. 1). The fibrils which compose this network are coarser around the periphery of the tubercle where they join the reticulum in the remains of the alveolar walls. The fibrils are situated between the cells and nowhere do they show any connection with the cells.

At twenty-eight days the tubercles have increased in size (Fig. 2). The outline of the alveoli involved can be followed. The fibrils which make up the network of reticulum are coarser than in the preceding stage, especially around the periphery of the tubercle; only a few of the latter appear in the photomicrograph. The cellular contents of the tubercle consists of mononuclear leucocytes and a few scattered lymphocytes. A few of the mononuclear leucocytes show slight necrosis, but there is no evidence of beginning caseation.

At fifty-six days the amount of reticulum has very materially increased (Fig. 3). The cellular contents of the tubercle is the same as in the twenty-eight day tubercle. Only the central portion of the tubercle appears in the photomicrograph. The tubercle as a whole is surrounded by bundles of coarse fibers which begin to show here and there the characteristic reaction of collagen to the silver impregnation. No caseation is present.

The next stage in the reparative process was found in the human lung, and all the succeeding stages are taken from the same source. The tubercle occurred in the lung of a negro child 9 weeks old who had been under observation for six weeks. The photomicrograph is taken from the center of the tubercle (Fig. 4). The tubercle is made up of mononuclear leucocytes and lymphocytes. The lymphocytes are situated around the periphery of the tubercle, while the mono-

THE RETICULUM OF THE LUNG*

IV. ITS PRESENCE IN THE REPARATIVE PROCESS OF THE TUBERCULOUS LESION WITH AND WITHOUT CASEATION

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The occurrence of reticulum as a distinct type of connective tissue and its presence in normal tissues was first demonstrated by Mall.³ By means of digestion experiments and chemical reactions he was able to differentiate it completely from elastic tissue, but found that it had many characteristics which showed that it was closely related to collagenous tissue. It will appear later that this has an important significance.

That reticulum is present in tuberculous lesions has been stated by numerous investigators; its demonstration, however, by special stains is due to the researches of Russakoff,⁶ Miller,⁵ and Foot.² Unfortunately no illustrations accompany the paper by Russakoff,⁶ but the papers of Miller⁵ and of Foot² contain numerous illustrations.

In his brief account of reticulum in tuberculosis, Russakoff⁶ pays more attention to the transformation of reticulum fibers into collagenous fibers, and their possible origin, than to the tuberculous process.

Miller, in his various contributions, has illustrated with photomicrographs the relation of reticulum to various tuberculous lesions and, like Russakoff, found that it is a precollagenous type of tissue. He also found that the fibrils of reticulum are intercellular in their position, that they are not continuous with any cells but are associated with large mononuclear leucocytes which, as shown by Foot,² have been described by some twenty different names.

Foot² studied the formation of reticulum in experimental tuberculosis and, like Russakoff and Miller, found it to be a precollagenous tissue which appears to be "a product of preëxisting reticulum, as it is always continuous with it."

It is the purpose of the present communication to show that reticulum plays an important part in the reparative process of tuberculous lesions even after caseation has taken place.

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sorbed. Extending through the caseous mass there is a large amount of reticulum which is but faintly shown in the photomicrograph. The tubercle itself is surrounded by a well marked band of collagenous fibers.

The lung, of which a small portion is shown in Fig. 7, was removed from a man 56 years old. He had been under observation for four years. The X-ray picture and the physical findings remained unchanged. It presents interesting features in connection with the reparative process in cases of pulmonary tuberculosis in which caseation has taken place.

A section through a portion of Fig. 7, stained with hematoxylin and eosin, is shown in Fig. 8. Under low power the tubercles (1 to 6) appear similar and take the rose-red tint of hyaline collagenous tissue but when impregnated with silver, a marked differentiation is brought out. In Fig. 8 the tubercles 1 and 6 appear to be confluent, but the silver impregnation shows that the slight depression above tubercle 1 marks out the boundary between the two tubercles. The five following photomicrographs are taken from the center of the tubercles and show transitional stages from pure reticulum to pure collagenous tissue.

There is so slight a difference between the tubercles marked 1 and 2 in Fig. 8 that they may be considered together. In each tubercle the center is made up of a network of reticulum (Fig. 9) which is rather more compact in tubercle 2 than it is in tubercle 1. Around the periphery of each tubercle there is a narrow band of collagenous fibers, while between the center and the periphery there are various gradations between fibrils of reticulum and collagenous fibers.

In tubercle 3 (Fig. 8), the center of the tubercle (Fig. 10) is made up of a network of coarse fibers of reticulum which almost immediately join still coarser fibers which show here and there the characteristic reaction of collagen to the silver impregnation. These latter fibers join the band of collagenous fibers which surrounds the tubercle. The band of collagen is more pronounced in this tubercle than in the others, due to the plane of section.

Fig. 11 is a section through tubercle 4 (Fig. 8), which gives the impression that it includes two alveoli. Possibly this is the case, for undoubtedly all the tubercles in the section are conglomerate. This appearance is the result, in this particular instance, of the photomicrograph including more of the left side of the tubercle than it does

nuclear leucocytes are streaming towards the center. There is slight necrosis of a few of the mononuclear leucocytes near the center of the tubercle. The reticulum has become coarser than in the preceding figures, and around the periphery of the tubercle it has been converted into collagenous tissue. The reticulum is much more abundant than the figure shows, since only one plane can be photographed.

The final stage in the series showing the part that reticulum plays in the reparative process of tuberculous lesions in which caseation has not taken place, is from the lung of a white child, 10 years old, who died of tuberculous meningitis (Fig. 5). The network of reticulum, which forms the center of the tubercle, is much more compact than in any of the preceding stages. The coarse bundles of fibers seen in the upper part of the photomicrograph are collagenous, while in the lower part they show a transitional stage from true reticulum to pure collagenous fibers. The cellular contents of the tubercle is almost exclusively mononuclear leucocytes with a few scattered lymphocytes.

In the tubercles thus far described the reticulum has consistently increased in quantity and quality from a network of fine fibrils (Fig. 1) to a dense, compact network of coarse fibers (Fig. 5) which are gradually being converted into collagenous fibers. The ultimate ending of this metamorphosis is a small scar composed of collagenous fibers.

In none of the tubercles has there been any evidence of caseation and none of them has contained a giant cell. Medlar⁴ has shown, however, that giant cells are a late production and that they represent "small areas of caseation or of simple necrosis around or into which mononuclear leucocytes have wandered," and indicate that a reparative process is taking place. In those instances in which slight indications of necrosis of some of the mononuclear leucocytes appear, there is an absence of polymorphonuclear leucocytes which are always associated with caseation and, since in every instance the network of reticulum has not been destroyed, there is evidence that no serious damage has been done and that the reparative process has become well established.

The tubercle shown in Fig. 6 is from the same lung as Fig. 5. This is an example of a tubercle in which caseation had commenced, but had been arrested before liquefaction had started, and reparation was taking place. The caseous material is being digested and ab-

In the second series, caseation complicated the reparation of the tubercle. With the arrest of the caseation, and after the necrotic tissue had been digested and absorbed, a new growth of reticulum filled the space thus created, and from this time on, the reparative process proceeded as in the first series; the result was a scar but, of necessity, larger than in the first series.

The statement is not infrequently made that collagenous fibers grow from the periphery of a tubercle into its interior, the inference being that they are present at the periphery from the initial period. In a young tubercle there are, as shown in Fig. 1, no collagenous fibers around its periphery but only fibers of reticulum. From these fibers prolongations extend into the interior of the tubercle where they unite to form a network.

As the reparative process progresses in a tubercle, the fibers of reticulum about its periphery increase in thickness and gradually become collagenous. If the center of a tubercle is not invaded by polymorphonuclear leucocytes in sufficient numbers to cause caseation and complete destruction of the network of reticulum, this reparative process is constantly repeated until the entire tubercle is converted into collagenous tissue (Fig. 13).

The transition from reticulum into collagenous fibers can be followed in sections impregnated by the silver method in which the fibrils of reticulum are black, the collagenous fibers are a golden or a brownish yellow, while the thickened fibers of reticulum which have not acquired collagen are of a more or less gray tint. The factors which lead to the conversion of reticulum into collagenous fibers are, to me, unknown.

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of the right, in order that the increased number of collagenous fibers could be shown to better advantage. In the photograph, the center of the tubercle is that part which appears to be made up of a more open network than the remaining portion. This is due to the fact that in the central part there is still a remnant of the network of fine fibers of reticulum, while the remainder of the tubercle is made up of collagenous fibers. As can be readily seen, the majority of the fibers are cut obliquely.

Tubercle 5 (Fig. 8), appears to be made up entirely of interlacing bands of collagenous fibers (Fig. 12), but in a few places, notably in the circular area in the lower right quadrant, remnants of the network of reticulum can be seen. With the exception of these scattered areas, all the fibers contain collagen and take the characteristic yellow color imparted by the silver impregnation.

In Fig. 13 from the center of tubercle 6 (Fig. 8) no remnant of reticulum remains; the entire tubercle has been converted into collagenous tissue that joins the scar situated immediately above it. In time tubercle 1 would have been converted into collagenous tissue and the entire mass would have formed a compact scar.

In this last series caseation had already begun, but had been arrested (Fig. 6). Fibrils of reticulum had extended from the band of collagenous fibers which surrounded the tubercle into the caseous area, and mononuclear leucocytes were streaming into the caseous material. Some of the reticulum had become fragmented, due in all probability to the proteolytic enzyme of the polymorphonuclear leucocytes. These fragments, like the caseous material, would in time be absorbed. New fibrils of reticulum growing in from those present around the periphery of the caseation would replace them and, following the removal of the necrotic tissue, would fill the space occupied by the caseous material with a new growth of reticulum (Fig. 9). Both the reticulum already present and the newly formed fibrils become coarser, and the network they form becomes more compact as the reparative process progresses until finally the entire tubercle is converted into collagenous tissue (Fig. 13).

A comparison of the reparative process in the two series brings out some interesting results. In the first series, the reparative process was not complicated by necrosis, and the development of reticulum and its conversion into collagenous fibers was unimpeded; the result was a small, often insignificant, scar.

FIG. 11. From the center of tubercle 4, Fig. 8. With the exception of scattered fibers of reticulum in the lighter portion of the figure, the tubercle is made up of collagenous fibers. $\times 500$.

PLATE 71

FIG. 12. From the center of tubercle 5, Fig. 8. This is almost exclusively made up of heavy bands of collagenous fibers. Small fragments of the network of reticulum which have not been wholly converted into collagenous fibers can be recognized.

FIG. 13. From the center of tubercle 6, Fig. 8. No reticulum is present. The entire tubercle has been converted into collagenous tissue.

DESCRIPTION OF PLATES

PLATE 66

- FIG. 1. Rabbit's lung. Mononuclear tubercle fourteen days after intravenous inoculation. The outline of an alveolus is marked out by heavy fibers of reticulum. These fibers give origin to fine fibrils which extend throughout the tubercle and form a well marked network. The fibrils of reticulum are situated between the cells and have no connection with them. No polymorphonuclear leucocytes present. $\times 500$.
- FIG. 2. Rabbit's lung. Twenty-eight days after intravenous inoculation. The tubercle is larger and the fibrils of reticulum are coarser than in the fourteen-day tubercle. A few of the cells in the lower right quadrant show slight necrosis, but there is no evidence of caseation. $\times 500$.

PLATE 67

- FIG. 3. Rabbit's lung. Fifty-six days after intravenous inoculation. The increased amount of reticulum and the increase in size of the fibers is especially noticeable.
- FIG. 4. From the lung of a negro child 9 weeks old. The dark bands around the periphery of the tubercle are made up of collagenous fibers. A few cells near the center of the tubercle show slight necrosis. $\times 500$.

PLATE 68

- FIG. 5. From the lung of a child 10 years old. The entire center of the tubercle is occupied with a network of coarse fibers of reticulum. The fibers in the upper part of the figure are collagenous; those in the lower portions show transitional stages between reticulum and collagenous fibers. $\times 500$.
- FIG. 6. From the same lung as Fig. 5. Caseation had taken place, but had been arrested. Fragmentation of the reticulum is shown. The entire caseation is permeated with fibrils of reticulum which do not appear in the photomicrograph. The tubercle is surrounded with collagenous fibers, some of which appear in the upper right quadrant. $\times 500$.

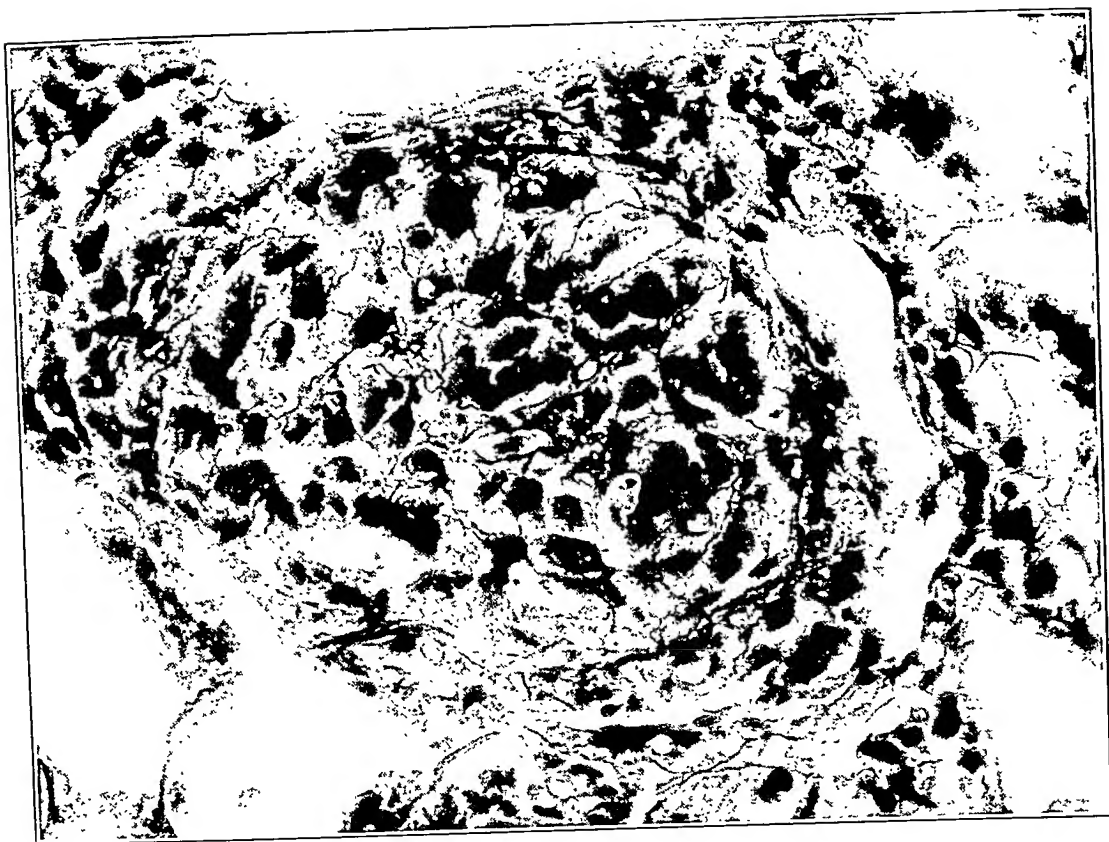
PLATE 69

- FIG. 7. Photograph of a portion of a lung which showed throughout its entire extent "healed" tubercles. $\times 1.5$.
- FIG. 8. Section from the lung shown in Fig. 7, stained with hematoxylin and eosin. The various tubercles numbered from 1 to 6, are shown in detail in Figs. 9 to 13. $\times 7.5$.
- FIG. 9. From the center of tubercle 1, Fig. 8. Shows a network of coarse fibers of reticulum. No collagenous fibers present. $\times 500$.

PLATE 70

- FIG. 10. From the center of tubercle 3, Fig. 8. The central portion is made up of coarse fibers of reticulum. The heavy fibers on either side are partly collagenous. $\times 500$.

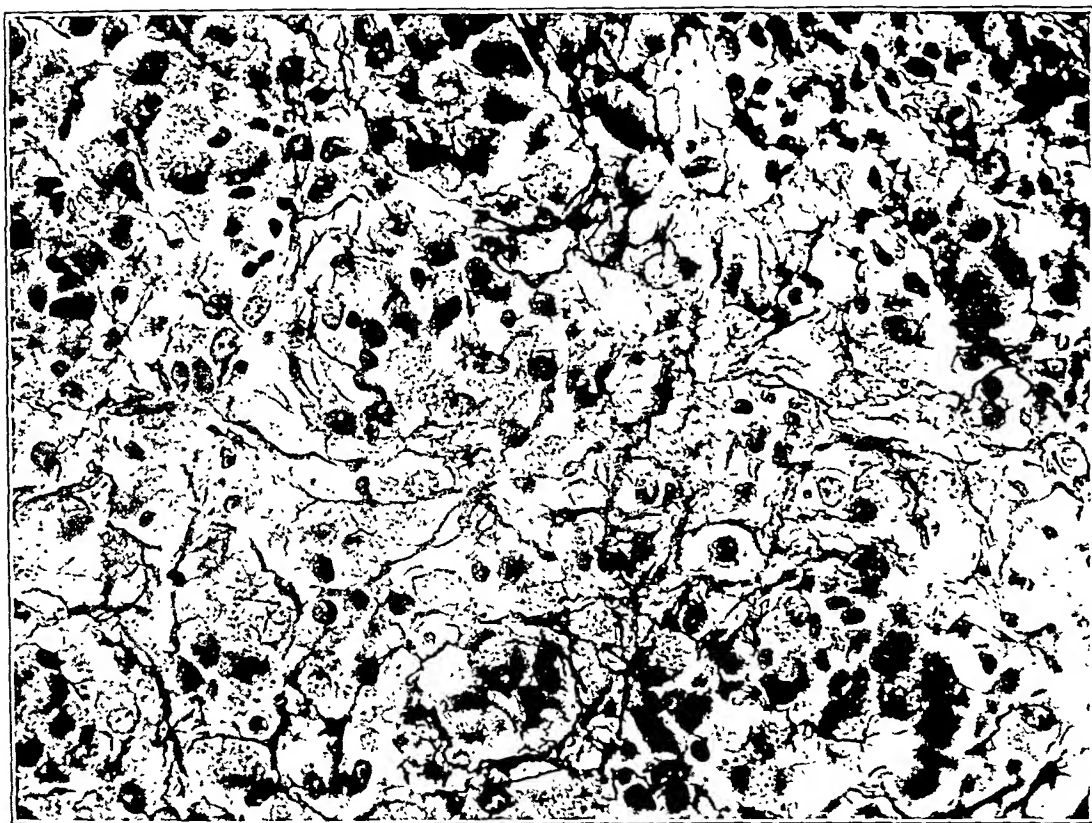




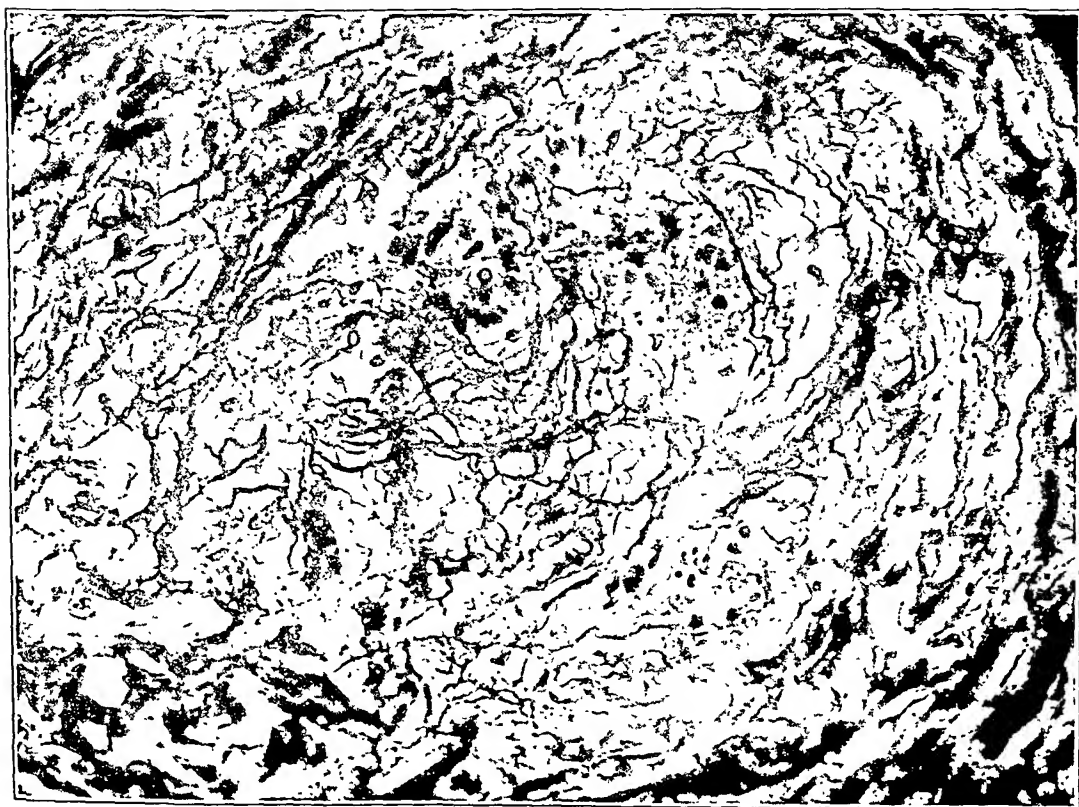
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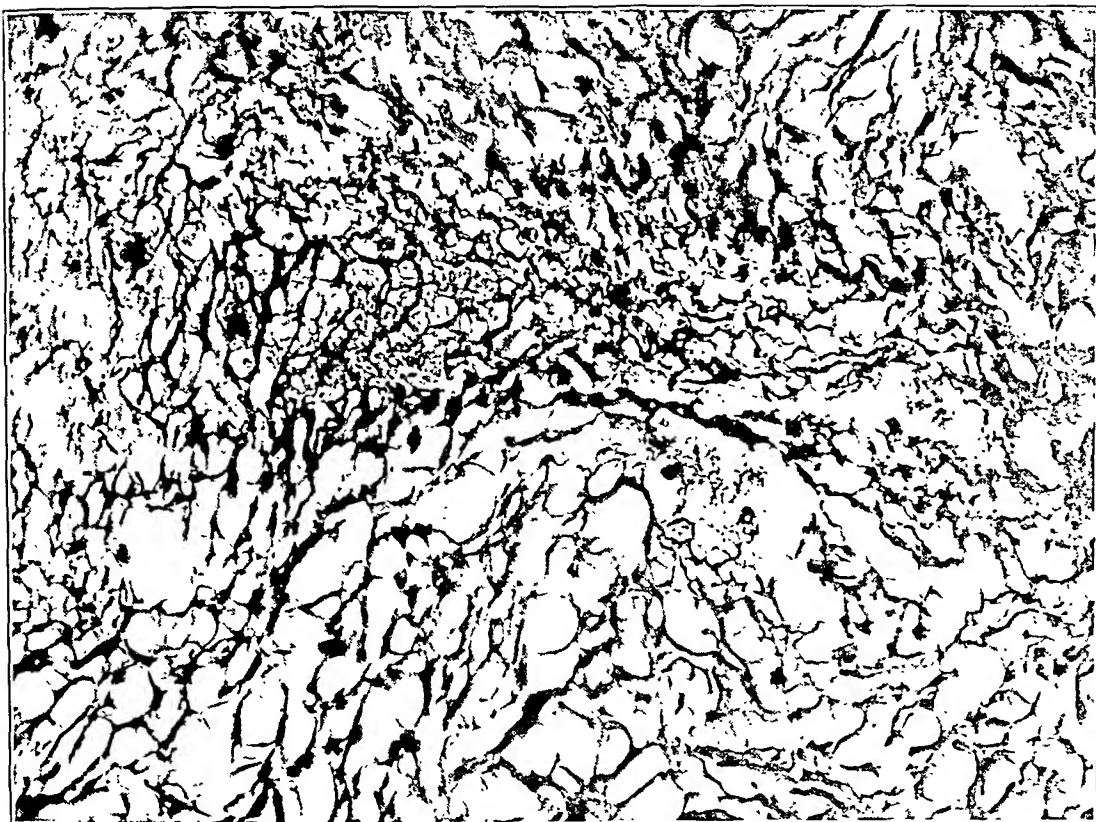
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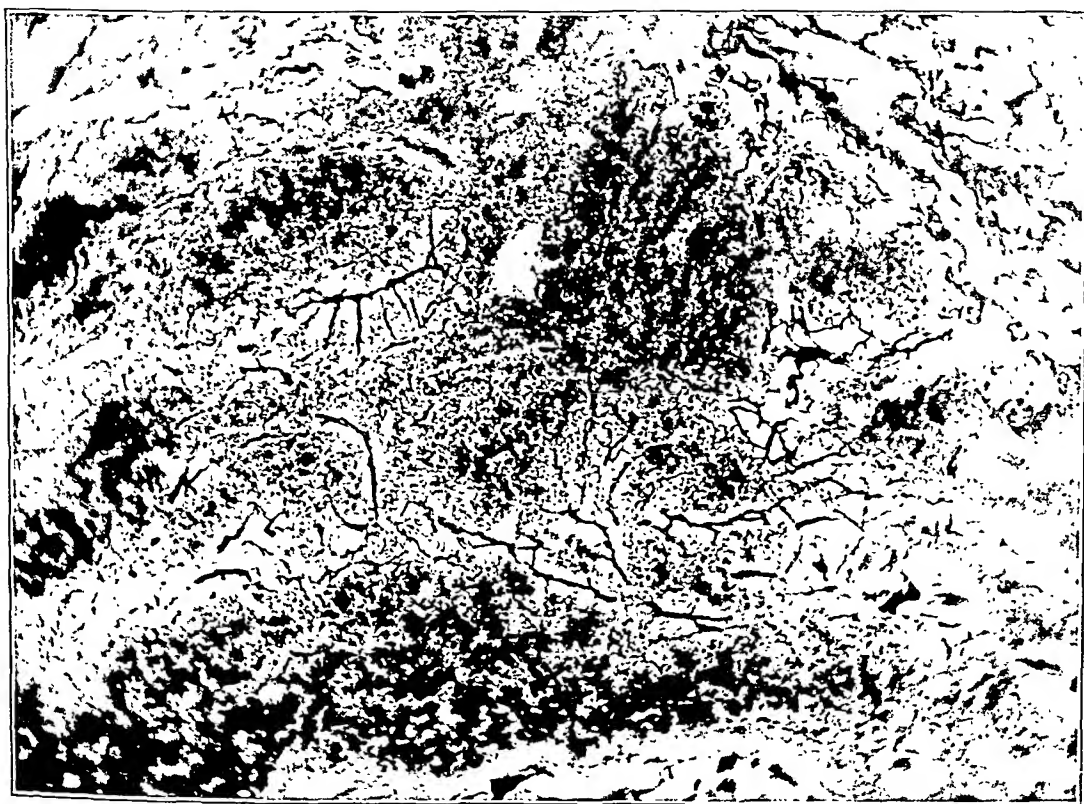
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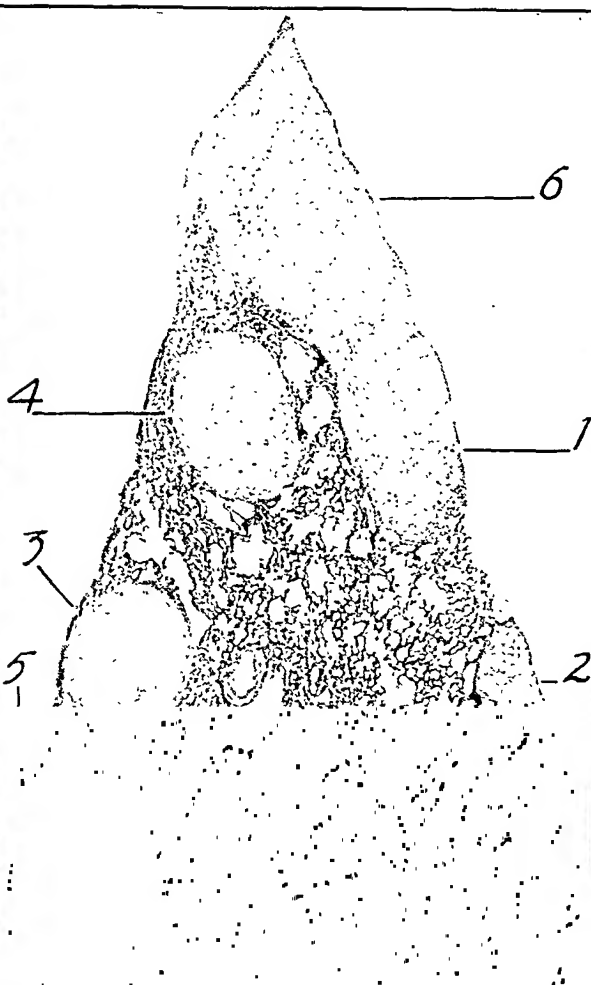
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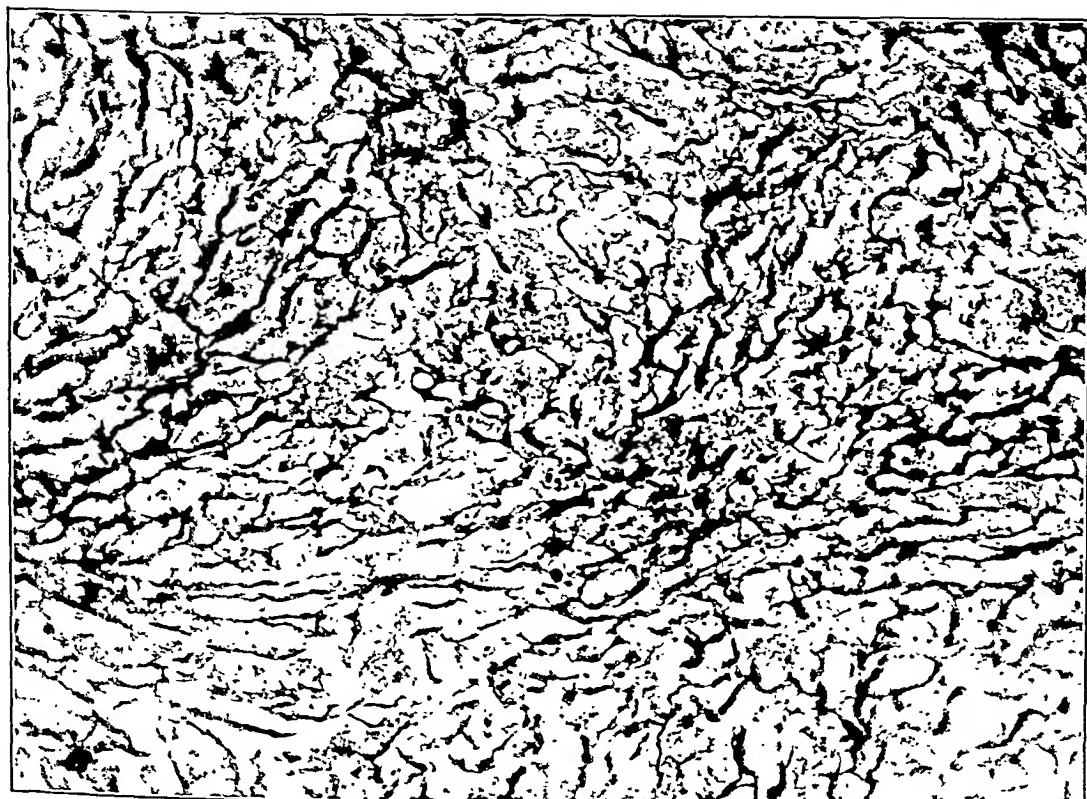
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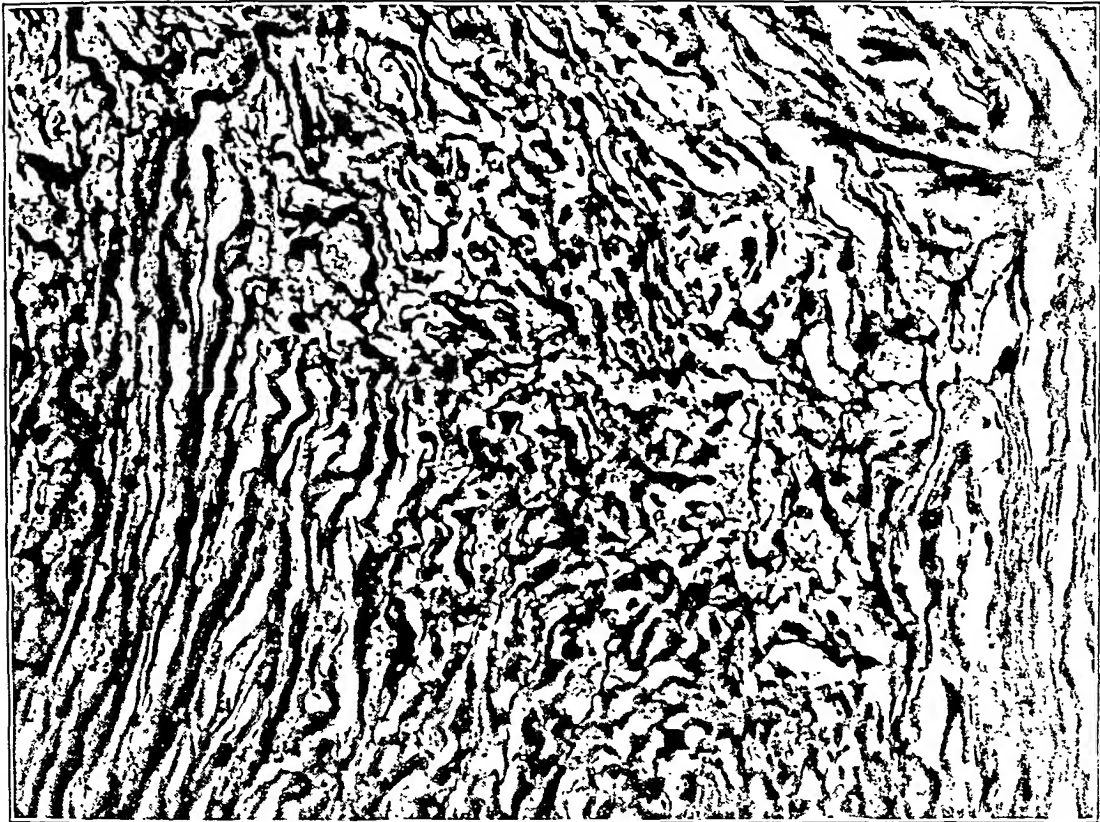
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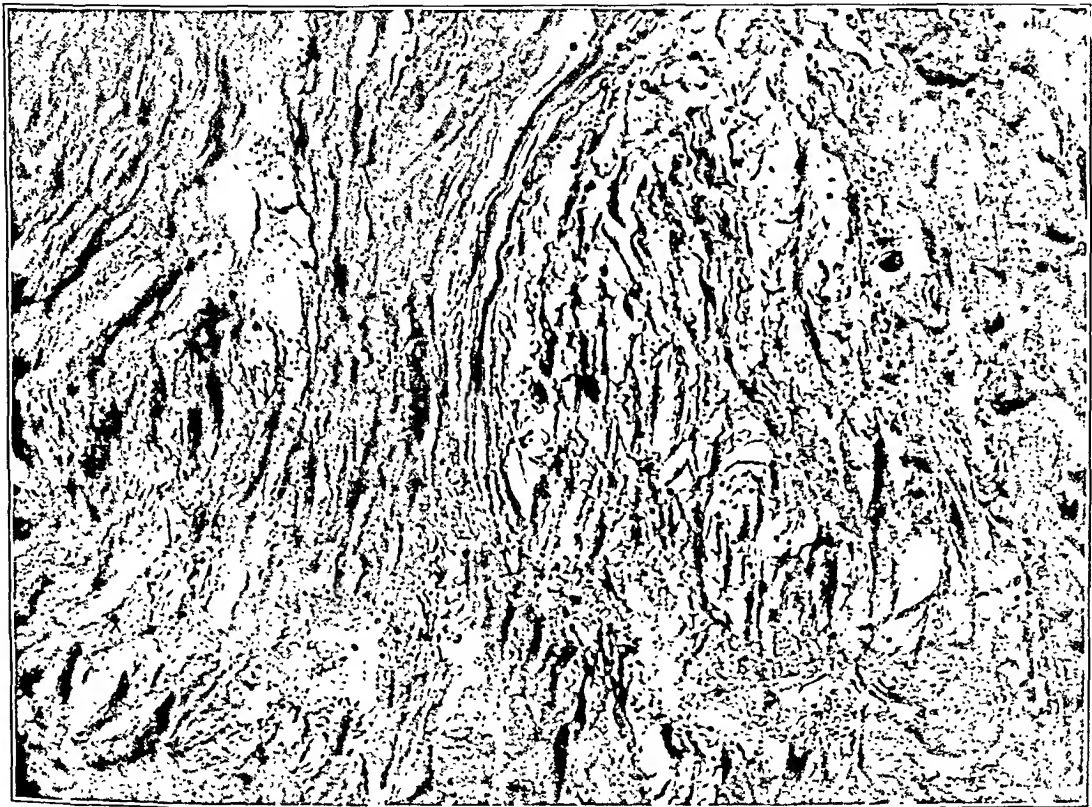
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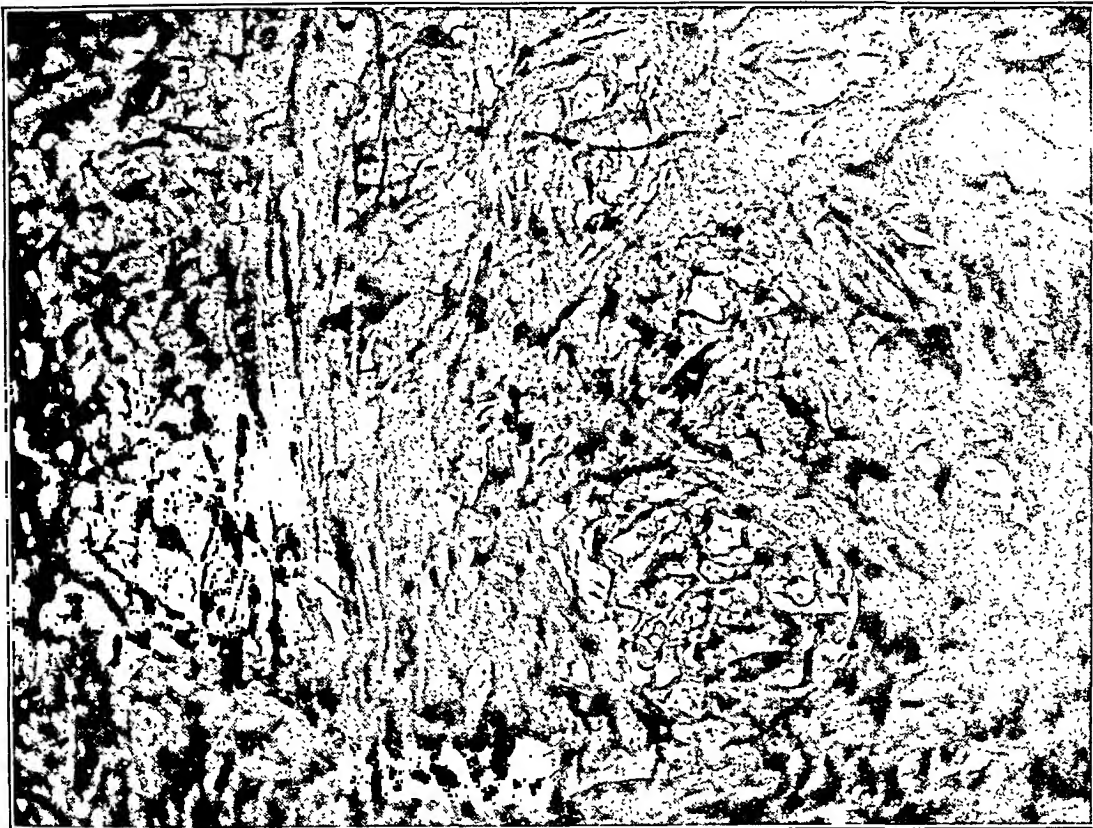
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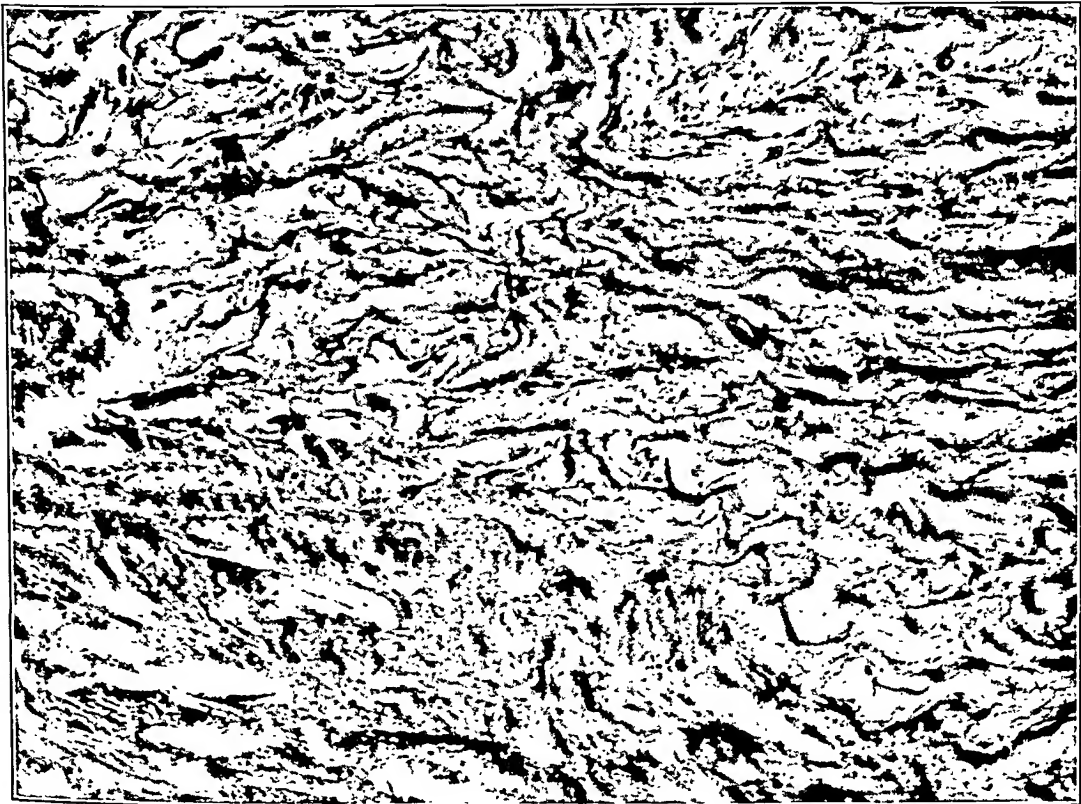
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and O'Farrell³ selected a monkey that showed the presence of mouth and laryngeal spirochetes, then injected pneumococci into the throat, and chilled the animal. It developed pneumonia and died, but an examination of the bronchi and lungs failed to reveal an invasion of spirochetes from the throat. In a second experiment they injected the sputum from a case of bronchial spirochetosis into the trachea of a monkey. In thirty-six hours it became ill, developed a high temperature and a cough, and mucus from the throat was laden with spirochetes. After two days the symptoms subsided, the spirochetes disappeared and the animal recovered. Kline⁶ has recently shown that gangrene may be produced experimentally by injecting spirochetes from a case of pulmonary gangrene into the traumatized tissues of guinea-pigs and rabbits, whereas gangrene is not produced in tissues that have not been subjected to trauma.

Several authors have found spirochetes similar to those described by Castellani, in the sputum and lung tissues of patients with pulmonary abscess or gangrene. It would seem, therefore, that these cases in varying stages comprise the chronic group described by Castellani. The diagnosis is often confused with tuberculosis due to the chronic cough, abundant foul-smelling sputum, emaciation, etc. The clinical picture of the acute form is not new as various authors would lead us to believe. Finding spirochetes in the sputum enhances our knowledge of the chronic cases only in so far as this secondary infection may mean a more prolonged and serious course with a greater tendency to gangrene. Foul-smelling sputum should always lead us to search for spirochetes. These organisms are usually overlooked in the routine examination of sputum, but are easily demonstrated when stained with a fresh solution of gentian violet, gentian violet followed with Gram's iodine, or better still, by the method of Warthin or Fontana.

Since we became interested in these spirochetes, we have studied the fresh sputum of at least a dozen patients with lung abscess or pulmonary gangrene, and along with the variegated bacterial flora present, we have always found a varying number of spirochetes and sometimes fusiform bacilli. In one case of lung abscess a small bronchial tree was coughed up and, when stained by the method of Levaditi, showed practically a pure culture of spirochetes. Sections of lung tissue from tuberculous and bronchiectatic cavities, where we expected to demonstrate spirochetes, have been studied but these

PULMONARY SPIROCHETOSIS *

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In 1905 Castellani¹ described a form of hemorrhagic bronchitis with numerous pleomorphic spirochetes in the sputum. To the condition he gave the name bronchial spirochetosis. Since that time numerous cases have been reported from various parts of the world, including twenty-four from the United States. There has been considerable discussion concerning the specificity of the spirochetes in these cases. Castellani,¹ Fantham⁹ and others described certain forms, varying in their morphology, of a species which they believe inhabit only the deeper air passages. Furthermore, certain characteristics are presented to differentiate these from the spirochetes found in the mouth and throat.

In a later report, Castellani² describes acute, subacute and chronic forms of the disease. Most of the subsequent writers have reported cases of the acute form, which is a mild disease and soon terminates spontaneously or responds quickly to the use of arsphenamine or tartar emetic.

These patients have cough, fever and the physical signs of bronchitis or broncho-alveolitis. The sputum is muco-purulent or blood-tinged and is laden with spirochetes. Castellani and others have found that the spirochetes in these cases vary in size and shape but may be separated into four types according to their morphology. In spite of this pleomorphism they believe that they may be differentiated from the spirochetes of the mouth by staining reaction, motility, etc. These authors have not mentioned the presence of fusiform bacilli. Fantham has described coccoid bodies which he thinks represent resting stages from which the spirochetes may develop.

All attempts to grow these organisms on artificial media have failed, and very little experimental work has been done. Chalmers

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left lung, but these were of recent formation and no interstitial pneumonia was present. A marked emphysema was evident in the portion of this lung which was not occupied by the abscesses.

No spirochetes had been found in the smears of the sputum or of the pus from the drainage wound in the chest, but after necropsy these smears were obtained and, after being restained, numerous spirochetes were found. Smears from the abscesses showed innumerable spirochetes. Sections from the walls of the abscesses were stained by the Levaditi method and showed various organisms in the necrotic exudate on the walls of the cavities, while in the deeper tissue and even invading the fibrous tissue, numerous spirochetes were found. In these areas no fusiform bacilli were seen in association with the spirochetes, but in the superficial areas some of the large organisms present might be suspected of belonging to this group.

Anatomic Diagnoses. Multiple abscesses, chronic interstitial pneumonia and bronchiectasis involving the entire left lung. Recent abscesses in the upper and lower lobes of the right lung and in the superior mediastinum. Chronic fibrous pleural adhesions obliterating the left pleural cavity. Recent fibrous adhesions at the apex and posterior surface of the right lung. Emphysema of the right lung. Thoracotomy with rib resection in the left axillary line. Marked lymphadenitis of the peribronchial nodes. Subacute splenic pulp reaction. Marked fatty infiltration of the liver. Moderate hydropericardium. Extreme emaciation.

CASE 2. Clinical Record. A man, aged 58, entered the Medical Service of the University of California Hospital, complaining of cough, fever and headache. He was subject to frequent colds, which he thought usually settled in the left chest causing pleurisy pains. A hacking cough had been present for about seven years which he supposed was due to excessive smoking. Three weeks before admission he had a head cold and then developed fever, a severe cough and a moderate amount of sputum. His cough and fever had persisted but his sputum had diminished in amount. A physical examination showed dullness at the base of the left lung posteriorly. The breath sounds were distant and bronchial in type. No râles were heard. The hemoglobin was 75 per cent, white blood cells 23,000, red blood cells, 3,800,000. X-ray examination of the chest showed a discrete mass in the lower portion of the left hilum 5×8 cm., and the base of the left lung diffusely gray with coarse mottlings. Bronchoscopy showed a nodular constricting mass at the bifurcation of the left bronchus. Throughout the course of five months in the hospital the patient ran a septic fever from 98.6 to 102.5 F. He finally developed a left-sided, slightly sanguinous pleural effusion of high specific gravity, and shortly before his death, began to expectorate a large amount of foul-smelling purulent sputum.

organisms were not found. In practically every case of pulmonary abscess or gangrene, however, which came to necropsy we have been able to demonstrate the organisms in abundance in the lung tissue.

The following illustrative cases have been studied during the past two years in the Pathological Department of the University of California Medical School.

CASE RECORDS

CASE I. Clinical Record. A girl, aged 12, was admitted to the Pediatric Service of the University of California Hospital, with a draining chest wound following a rib resection for a post-operative pneumonia and empyema. Two years before admission she had her tonsils and adenoids removed, and shortly afterward, developed pneumonia of the left lung with empyema. One month later a rib resection was done and pus drained from the left pleural cavity. Ten months later, sections of four ribs were removed and the lung collapsed. The wound has drained since that time, and later, a physical examination showed a markedly emaciated, anemic, dyspneic child with a very foul breath and a foul discharge from the wound in the left chest. The entire left chest and right apex were dull to percussion. No breath sounds were heard over the left lung. The edge of the liver was felt well beneath the costal margin. The fever ranged about 102 F. The X-ray diagnosis was probable gangrene of the left lung. The child became apathetic, and died five days after admission to the hospital.

Pathologic Record. Left lung: weight 400 gm. The left pleural cavity was practically obliterated by thick, fibrous adhesions. Very large edematous lymph nodes were found at the hilum. The pulmonary artery was free from thrombi. The entire lung was firm and boggy with no suggestion of crepitation. Section showed multiple abscesses in the upper lobe, varying in size from 3 mm. up to 1.5 cm. in diameter, and filled with foul-smelling purulent material. A diffuse bronchiectasis was present. Between the abscesses there was a chronic interstitial pneumonia with an overgrowth of a dense, boggy fibrous tissue. Most of the lower lobe was occupied by a large ulcerating cavity divided into numerous compartments and containing a foul purulent material. The walls of this cavity showed an irregular, necrotic surface.

Right lung. Weight 240 gm. Recent fibrous adhesions had formed at the apices of the upper and lower lobes. The upper half of the upper lobe contained a large, thin-walled abscess which communicated with a similar abscess in the superior mediastinum. In the apex of the lower lobe there was another large abscess. All of these abscesses contained foul, purulent material like that found in the

Right lung. There were several small abscesses towards the apex and larger gangrenous abscesses near the base. The intervening lung tissue showed a marked chronic interstitial pneumonia with fibrosis, and was diffusely mottled with yellowish dots indicating the presence of fat-laden phagocytic cells. The abscesses contained a greenish yellow, foul-smelling pus, and had tags of necrotic tissue on their walls. Smears from these abscesses showed numerous spirochetes and a few fusiform bacilli. Sections stained by the Levaditi method showed a large number of spirochetes in the lung tissue.

CASE 4. Clinical Record. A Chinese male, aged 39, entered the Medical Service of the San Francisco Hospital complaining of a bilateral chest pain and an irritating cough. Two months before entry he caught a cold, developed a fever and at times became delirious. One month ago he became worse, began to cough and raise considerable foul-smelling sputum which, at times, contained lumps of yellowish material. Hemoptysis occurred once. He recently developed bilateral chest pain. Physical examination showed marked emaciation, dullness at the bases of both lungs with flatness and other evidence of fluid at the right base. Moist râles were heard over both bases. The liver was enlarged and the fingers showed marked clubbing. The blood count was 3,088,000 erythrocytes, and 23,900 leucocytes. The fever was septic in type ranging from 100 to 104 F. The sputum was not examined for spirochetes but was negative for tubercle bacilli.

Pathologic Record and Anatomic Diagnoses. Large gangrenous abscess in the lower lobe of right lung communicating with an encapsulated empyema. Marked chronic interstitial pneumonia of right lung. Marked bronchiectasis of right lung, moderate in the left lung. Bronchopneumonia in both lobes of the left lung. Fibrous pleural adhesions obliterating the right pleural cavity. Few fibrous adhesions about the left lung.

Right lung. Weight 1270 gm. Thick, fibrous pleural adhesions covered the entire surface. Between the lower lobe and the thickened pleura there was an encapsulated empyema with foul-smelling greenish pus, which communicated with a large gangrenous abscess in the lower lobe. In the upper lobe there was thrombosis of a large vessel. Large bronchiectatic cavities occurred in all lobes, and there was marked interstitial pneumonia with no crepitant areas in the entire lung.

Left lung. Weight 780 gm. No abscesses were found, but there was slight bronchiectasis and bronchopneumonia in the lower lobe.

Smears from the abscesses in the right lung were stained by Warthin's method, and showed innumerable spirochetes and a few large

Pathologic Record and Anatomic Diagnoses. Primary carcinoma of the left bronchus with a large mass extending into the substance of the lung and into the lymph nodes at the hilum. Extensive metastases to the liver. Gangrenous bronchiectasis of the entire lower lobe of the left lung with ulceration of the surface, and a large encapsulated empyema between the lower lobe and the thickened pleura. Interstitial pneumonia, in the upper lobe of the left lung and in the all lobes of the right lung. Fibrino-purulent pleuritic exudate over the posterior surface of the right lung. Extensive fibrous pleural adhesions over both lungs. Marked hypertrophy and dilatation of the right ventricle.

The sputum and fluid obtained by thoracentesis had not been examined for spirochetes, but smears obtained from a gangrenous area in the lower lobe of the left lung showed numerous spirochetes. Sections stained by the Levaditi method also showed spirochetes in the gangrenous lung tissue.

CASE 3. Clinical Record. A young man, aged 18, was admitted to the University of California Hospital complaining of a persistent cough and much sputum. Two months before admission he had pneumonia with a right-sided pleurisy. He was in bed only a week and had apparently recovered, but four weeks later a sharp pain suddenly developed low in the right chest at the site of the previous pleurisy. A productive cough developed and he raised a large amount of greenish yellow, foul-smelling, purulent sputum. Physical examination showed a very foul breath, dulness to flatness at the base of the right lung and an area of hyper-resonance with amphoric breathing and bubbling râles. There was a positive Grocco's sign on the left side. The blood count showed 3,400,000 red cells and 12,700 white cells. The sputum was foul, yellowish green, and measured about 400 cc. in twenty-four hours. Smears showed pus cells, elastic tissue, streptococci and staphylococci, but no acid-fast organisms. No note was made regarding spirochetes. X-ray examination showed a shadow obliterating the right base and areas of rarefaction extending upwards and mesially. The whole lung above showed a mottled grayness especially extending from the hilum to the apex. Nine days after admission, portions of the eighth and ninth ribs were resected in the right axillary line. Dense pleural adhesions were encountered, and a small abscess cavity was found in the lower lobe. The patient died several hours after the operation.

Pathologic Record and Anatomic Diagnoses. Multiple abscesses of right lung with marked chronic interstitial pneumonia and bronchiectasis. Fibrous pleural adhesions obliterating right pleural cavity. Bronchopneumonia in the lower lobe of the left lung. Emphysema of the left lung. Recent thoracotomy with rib resection in right axillary line.

COMMENT

From a study of these cases it seems evident that we are not dealing with a new clinical entity. The histories and necropsy findings suggest that certain serious pathologic conditions would have been present in these lungs if they had never become infected with spirochetes. It would seem, therefore, that there is always some primary destructive lesion followed by a secondary invasion by these organisms. In the study of lung abscesses and pulmonary gangrene, they have probably been overlooked because they stain poorly or not at all by ordinary staining methods. On the other hand it is probably of some significance that they are present in large numbers and are found more deeply seated in these lesions than other organisms. Just as Vincent's angina is a serious complication of acute tonsillitis, so the presence of these organisms in the lung tissue may mean more serious lung destruction by abscess or gangrene.

The organisms demonstrated in the above cases are variable enough in their morphology to correspond to all the types described by Castellani. The question then arises as to whether bronchial spirochetosis as an acute, subacute or chronic disease, is ever a clinical entity; that is, are these spirochetes ever the primary cause of such symptoms or, are they always secondary invaders? From a study of the chronic cases we find no evidence to oppose the belief that they are always secondary to some other infectious or destructive lesion. Symbiosis with other organisms probably plays no part in their growth, for they seem to thrive with a heterogeneous group of organisms in tissue that has been previously injured or is undergoing destruction. The next questions that arise are, is there any relationship between these spirochetes and the ones found in the human mouth, and is there any relationship between these organisms and the ones found in Vincent's angina? These questions we cannot definitely answer. The majority of those found in lung tissue are more slender and have more waves than those found in the mouth, although some do resemble the refringens type. Likewise these usually have more spirals than the Vincent type, but this type is encountered and organisms resembling fusiform bacilli are seen frequently enough to suggest that they are similar infections if not the same. The subject is open for further research to determine the relationship of the different types of spirochetes, and their significance in the various inflammatory processes in which they occur.

organisms that were probably fusiform bacilli (Fig. 3). Sections from the wall of these abscesses also showed numerous spirochetes in the tissue when stained by the Levaditi method.

CASE 5. Clinical Record. A man, aged 61, entered the San Francisco Hospital complaining of cough and aphonia. He had been coughing and raising large quantities of sputum for about three years. He never had hemoptysis. About two months before admission he caught cold during the night and completely lost his voice. The cough, with large amounts of sputum and a daily high fever, persisted and he became very weak. Physical examination showed marked dyspnea, teeth carious and advanced pyorrhea, and extreme emaciation. The left chest showed limitation of movement. There was dullness over both apices with many moist râles at the apices and bases. At the left apex signs of cavity formation were found. Examination of the heart was negative. Wassermann tests were repeatedly negative. There was a leucocytosis of 15,000 with 58 per cent polymorphonuclears. The X-ray examination suggested tuberculosis of the left lung, and upper lobe of the right lung. The patient had a septic temperature and raised large amounts of foul-smelling sputum during his three weeks in the hospital. Examination of the sputum failed to show tubercle bacilli, and no note was made regarding the presence of spirochetes. The day before his death he developed signs of acute pulmonary edema and failed to respond to treatment.

Pathologic Record and Anatomic Diagnoses. Multiple abscesses in the upper lobe of left lung associated with bronchiectasis, fibrosis and interstitial pneumonia. Diffuse purulent bronchopneumonia of both lungs. Fibrous pleural adhesions over upper lobe of left lung. Emphysema of both lungs. Marked atherosclerosis. Aneurysmal dilatation of the arch of the aorta. Hypertrophy of the right ventricle. Marked hyperplasia of the peribronchial and mediastinal lymph nodes.

Left lung. Weight 850 gm. The upper lobe had multiple cavities filled with dirty, foul-smelling pus. Several large, greenish areas of necrosis with beginning cavitation were seen. Smaller cavities filled with yellow creamy pus were found in the lower lobe. Interstitial pneumonia, diffuse bronchiectasis and extensive patches of necrotic, purulent bronchopneumonia occurred in both lobes.

Right lung. Weight 600 gm. Fibrous adhesions had formed at the apex and base. Purulent bronchitis and small areas of bronchopneumonia occurred in all lobes, and there were a few small abscesses in the lower lobe.

Smears from the abscesses in both lungs contained numerous spirochetes, and sections stained by the Levaditi method showed an abundance of organisms in the lung tissue.

CONCLUSIONS

1. Spirochetes are frequently found in the sputum and lung tissue of patients with lung abscess or gangrene.
2. The frequent association of fusiform bacilli with these organisms suggests that they may be of the Vincent type, and not a definite entity as described by Castellani.
3. These organisms are readily demonstrated in the sputum when stained by gentian violet and Gram's iodine, or by the Warthin method, and in the tissues by the Levaditi or Warthin method.
4. Proper oral hygiene as a prophylactic measure, and treatment with arsphenamine in early recognized cases may be of benefit.
5. All forms of pulmonary spirochetosis probably represent a secondary infection. Their presence in large numbers, deeply situated in the tissue suggests some pathogenic influence.

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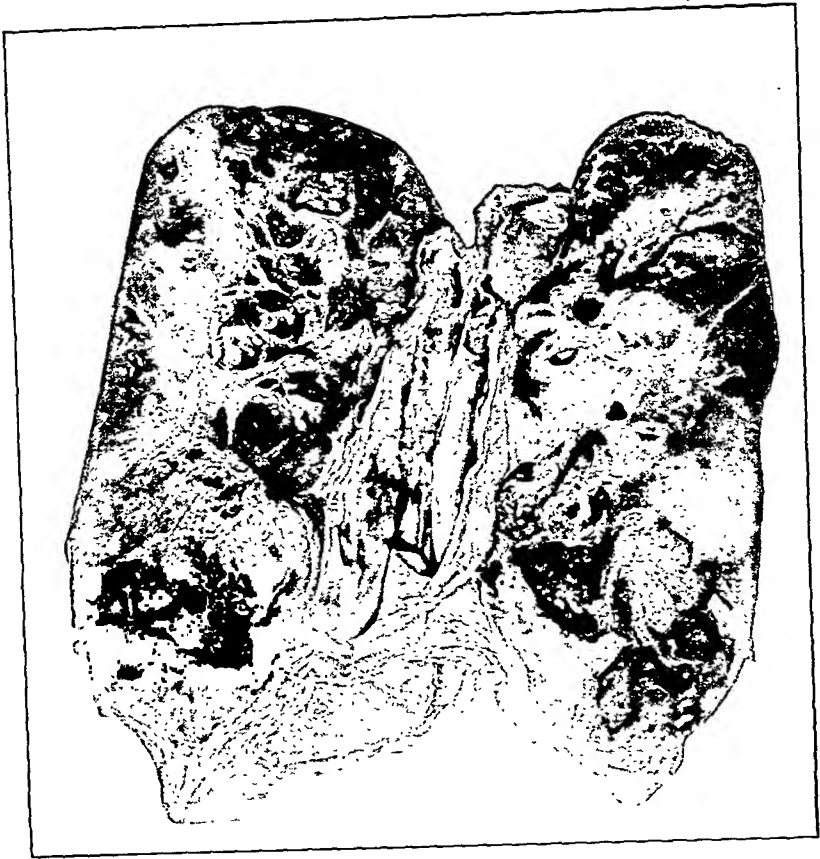
DESCRIPTION OF PLATES

PLATE 72

- FIG. 1. Case 1. Left lung. Pulmonary abscesses and interstitial pneumonia.
 FIG. 2. Case 1. Right lung. Gangrenous abscesses.

PLATE 73

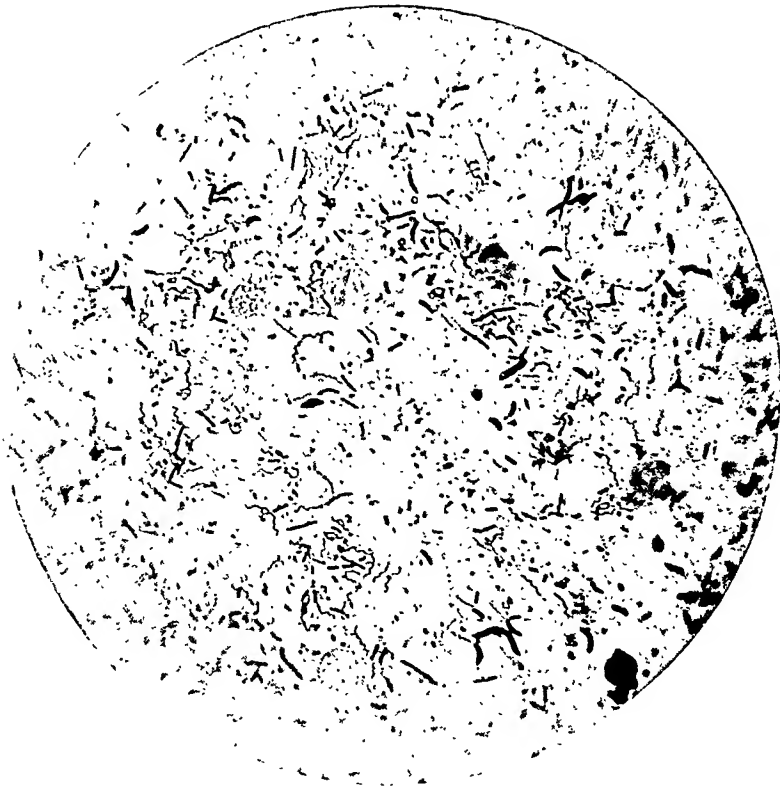
- FIG. 3. Case 4. Spirochetes in smear from gangrenous abscess of the lung.
 FIG. 4. Case 3. Multiple abscesses and interstitial pneumonia.



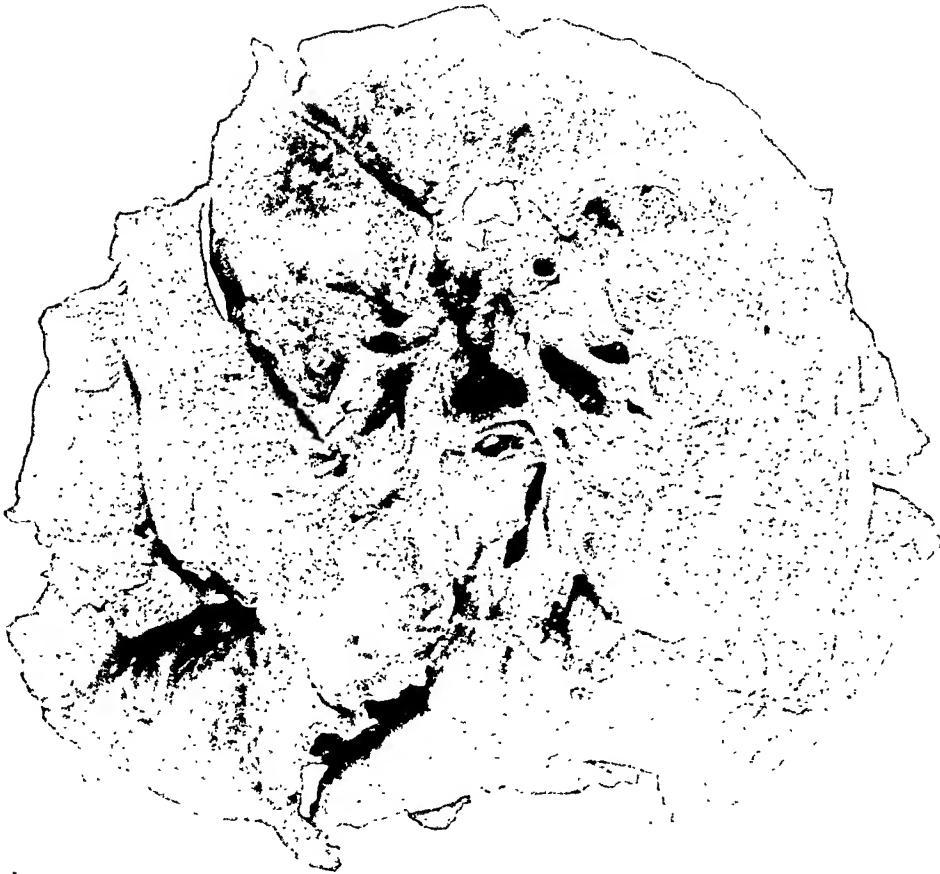
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Smith and Rusk

Pulmonary Spirochetosis

CLINICAL HISTORY OF THE RABBIT

In 1919 the rabbit began its career as the pet of a family with several children. It remained apparently healthy until a few months before its exodus. At this time the animal became listless, lost its appetite and became greatly emaciated. A few days before death, blood was passed from what was taken to be the rectum. A necropsy was performed because it was suggested that there was a possibility of a malignant tumor of the bowel or the pelvic viscera.

It is of interest that the animal was a virgin female that presumably had experienced neither trauma, pregnancy nor infection, factors which might be considered as predisposing to the development of a malignant tumor. The animal was $4\frac{1}{2}$ years of age. On Sept. 23, 1924, the animal was killed by chloroform and immediately a necropsy was performed.

NECROPSY REPORT

The animal is quite emaciated. The abdomen is greatly distended. Superficially no evidence of newgrowth is found. The peritoneal cavity contains about 500 cc. of straw-colored fluid. The lower abdomen is filled with a greatly distended bladder and two large irregular cystic masses which displace the intestines upward (Fig. 1). These masses conform to the Fallopian tubes which have been transformed into convoluted cystic masses measuring up to 16 cm. in diameter. These lobulations form a continuous cystic mass on either side with very thin walls but in the regions where the tubes join to form the uterus, the tissues are densely infiltrated with firm white nodules which completely replace the uterine tissue. The tumor tissue extends downward, infiltrating the vaginal walls but giving rise to no mucosal ulcerations. From the uterus the tumor extends into the bladder wall massively infiltrating the region of the trigone. As a result of this obstruction the bladder is greatly dilated to many times its normal size, measuring 15 cm. in diameter. The bladder wall is very thin, and extending from the region of massive infiltration are fine lines of tumor tissue invading the lymphatics. The bladder contains blood-tinged cloudy urine.

From the primary growth, the retroperitoneal, mesenteric, mediastinal and cervical lymph nodes are involved in the tumor invasion, forming a chain of masses from the pelvis to the neck varying in size from that of a bean to a lemon. On section the nodes can be seen to be composed of a uniform, opaque, whitish tissue evidently carci-

ADENOCARCINOMA OF THE UTERUS IN A RABBIT *

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Although rabbits are such common laboratory animals, the finding of spontaneous newgrowths among them, either benign or malignant, is quite rare. Bell and Henrici¹ were able to find reports of only thirty-five tumors. We have found reports of ten additional tumors. Of these forty-five tumors, twenty-four arose in the uterus. Stilling and Beitzke² state that in the course of their work from 1902 to 1913 they observed thirteen instances of tumors of the uterus. There were seven adenomas, four myomas and two apparently malignant tumors which infiltrated the wall of the uterus but gave rise to no distant metastases. Their attempts at transplantation into other rabbits failed to reproduce the tumor. Boycott³ reports four instances of uterine tumors which arose at the site of placental remains, infiltrated the wall of the uterus and extended into the broad ligament. These tumors gave rise to no distant metastases. Other authors report single instances of uterine tumors, Wagner,⁴ Selinow,⁵ Katase,⁶ Marie and Aubertin,⁷ Leitch,⁸ Shattock,⁹ and Lack.¹⁰

The next most common site for tumors in rabbits is in the kidneys. These tumors have all been of the benign type giving rise to no metastases. None of these tumors was successfully transplanted into other rabbits. Seven such tumors have been reported.

There are reports in the literature of spontaneous carcinomas arising in the lung, stomach, accessory pancreas and in the breast, while sarcomas have arisen in the subcutaneous tissue, the omentum and elsewhere.

Recently W. H. Brown and Louise Pearce¹¹ have reported an instance of carcinoma developing at the site of a scrotal syphilitic infection. The tumor recurred after removal and finally metastasized to many organs leading to the death of the animal. This accidental occurrence led them to undertake an extensive experimental study of the relation between syphilitic infection and the incidence of cancer.

* Read before the section of Pathology and Bacteriology at the annual meeting of the California State Medical Association.

Received for publication, January 6, 1926.

Bladder. The bladder wall is greatly thinned. The mucosal lining is intact and is composed of two to three layers of epithelial cells. The mucosa appears normal but the submucosa is very vascular and congested. The blood vessels are thin-walled. There are several clumps of cancer cells which have infiltrated the submucosa almost to its epithelial lining. These cells are enclosed in small spaces lined with endothelial cells. These spaces undoubtedly represent dilated lymphatics.

The muscular coat of the bladder is diffusely infiltrated with the newgrowth. There is a rather dense desmoplastic reaction accompanying the tumor. There is a tendency for some of the central cells to undergo necrosis.

Lung. The lung tissue is normal except for a slight amount of congestion. Scattered through the tissue are metastatic tumor nodules. Each of these is composed of a narrow rim of well preserved cancer cells in gland-like arrangement. Within this narrow rim of growing tissue the cells gradually lose their staining power and become necrotic. Microscopic collections of cancer cells are found elsewhere in the lung.

Kidney. There is a marked dilatation of the calices and the tubules. This distension is greatest in the collecting tubules and gradually decreases in the cortex. The epithelium of the dilated tubules is flattened. Between the tubules there is a marked interstitial proliferation of fibrous tissue. The pelvis contains a small amount of fibrino-sanguinous exudate. There are no cancer cells present.

Lymph Node. The architecture of the retroperitoneal lymph nodes is completely distorted due to an extensive infiltration by the newgrowth, which is accompanied by a rather dense fibrous stroma and assumes a medullary type of growth. Several dilated spaces lined with endothelium, evidently dilated lymphatic vessels, contain clumps of epithelial cells. Some of these cells are well preserved and have mitotic figures.

ATTEMPTS AT TRANSPLANTATION

Immediately following the autopsy attempts were made to transplant the tumor into six other rabbits. The methods used were the subcutaneous and intraperitoneal injections of finely-chopped tumor mixed with warm normal saline solution. Four of these rabbits subsequently died of infection, while two were killed with chloroform

noma. In the region of the apex of the right lung is a mass, 2 cm. in diameter composed of tumor tissue involving a lymph node.

The right kidney is negative, but the left is moderately enlarged and, on section, reveals a marked hydronephrosis. There is a small rather recent blood clot in the pelvis. The ureter on this side is dilated and is partially obstructed where it enters the bladder.

Metastatic nodules of varying size are present in the liver and lungs.

The heart, spleen, osseous tissue, gastro-intestinal tract and central nervous system are entirely free of newgrowths.

MICROSCOPIC EXAMINATION

Uterus. There is very little endometrium present. On the inner surface is a small area lined with a single layer of columnar cells. Immediately beneath this area is an edematous fibrous tissue containing vessels which are congested and thin-walled. In this region there is a dense infiltration of lymphocytes. There are small groups of epithelial cells growing in strands which have large vesicular nuclei with finely granular basophilic cytoplasm. Some of these have undergone necrosis.

The muscular coat is extensively broken up into irregular bands by an infiltration of the newgrowth with an accompanying desmoplastic reaction. In general this newgrowth has a glandular arrangement with alveoli of varying sizes. These glandular structures are lined with a single layer of small cuboidal cells with deeply staining nuclei and with very little cytoplasm. In some areas the alveoli are filled with hyaline, pink-staining material. The cells lining these dilated alveoli are greatly flattened. The growth is definitely adenomatous in some areas, while in others it has a papillary form. A few mitotic figures are present.

Fallopian Tube. The inner surface is lined with a layer of very flat epithelial cells. The tube is so stretched that the normal convolutions are obliterated. The tissue beneath the lining layer is a vascular and edematous stroma. This layer and the remaining coats of the tube are infiltrated with a newgrowth of epithelial cells which present the glandular and papillary arrangement much like that seen in the uterus. Mitotic figures are present. Sections of the tubes taken elsewhere show the wall to be greatly thinned in places and composed mainly of a hyaline fibrous material.

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DESCRIPTION OF PLATES

PLATE 74

FIG. 1. Shows the viscera at the time of autopsy. The bladder is greatly distended and shows dilated lymphatics in its wall containing carcinoma. The irregular lobulated cystic masses in the lower abdomen are the Fallopian tubes. Between the bladder and tubes, the uterus can be seen infiltrated with carcinoma which extends into the bladder and tubes. Metastases may be seen in the omentum, liver, right lung and at root of neck.

PLATE 75

FIG. 2. Microscopic picture of the uterus (low power) showing glandular type of growth with hyaline material within the alveoli. Also shows muscle bands between the cancer nodules.

FIG. 3. Uterus under 4. mm objective. Note the smooth muscle cells between the alveoli.

PLATE 76

FIG. 4. Bladder shows cancer cells in dilated lymphatics.

FIG. 5. Lymph gland shows invasion of the gland with carcinoma growing in solid masses. Mitotic figures are present.

PLATE 77

FIG. 6. Fallopian tube shows invasion of the entire wall with a papillary-adenomatous type of newgrowth.

FIG. 7. Lung showing metastatic nodule with central necrosis.

several months after inoculation. None of the rabbits showed tumor growth.

W. H. Brown and Louise Pearce were not successful in transmitting a cancer to other rabbits until they made an emulsion of the tumor cells and then injected them into the testicles of other rabbits. The emulsion was made by grinding some of the tumor tissue in a mortar and adding normal saline solution, 0.5 cc. being used as the dose. They succeeded in transmitting the tumor in this way to a number of rabbits.

SUMMARY

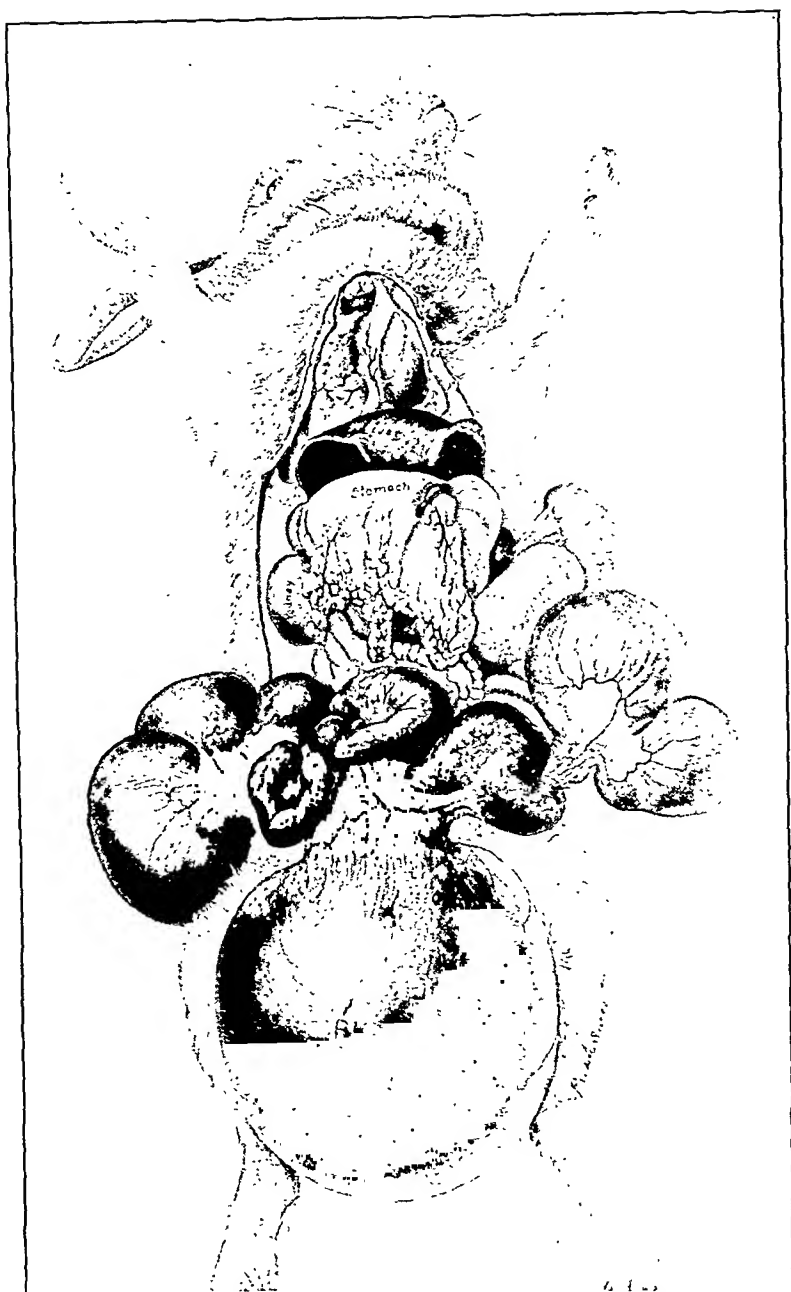
A virgin female rabbit, $4\frac{1}{2}$ years of age, developed a spontaneous carcinoma of the body of the uterus with extension into the tubes, bladder and vaginal walls. Extensive metastases were formed in the retroperitoneal, mesenteric, mediastinal and cervical lymph nodes, in the liver and lungs.

The carcinoma in general maintained an adenomatous or papillary adenomatous type but in some areas it grew in a medullary form. In some areas the tumor alveoli contained a colloid-like material. The tumor cells could be seen invading the dilated lymphatics of the bladder and lymph nodes.

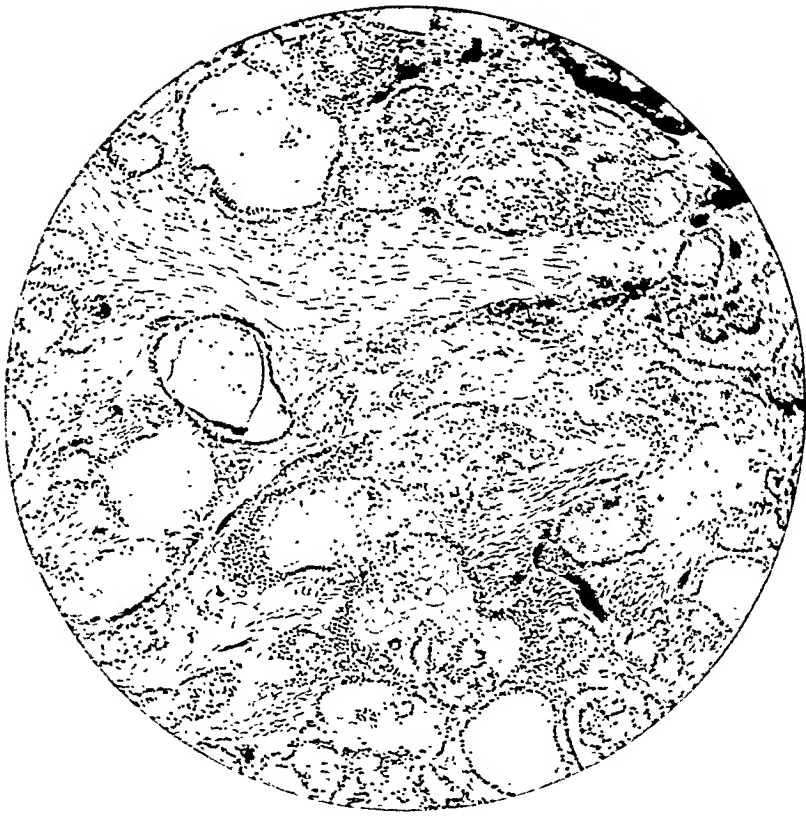
All efforts at transplantation failed to reproduce carcinoma.

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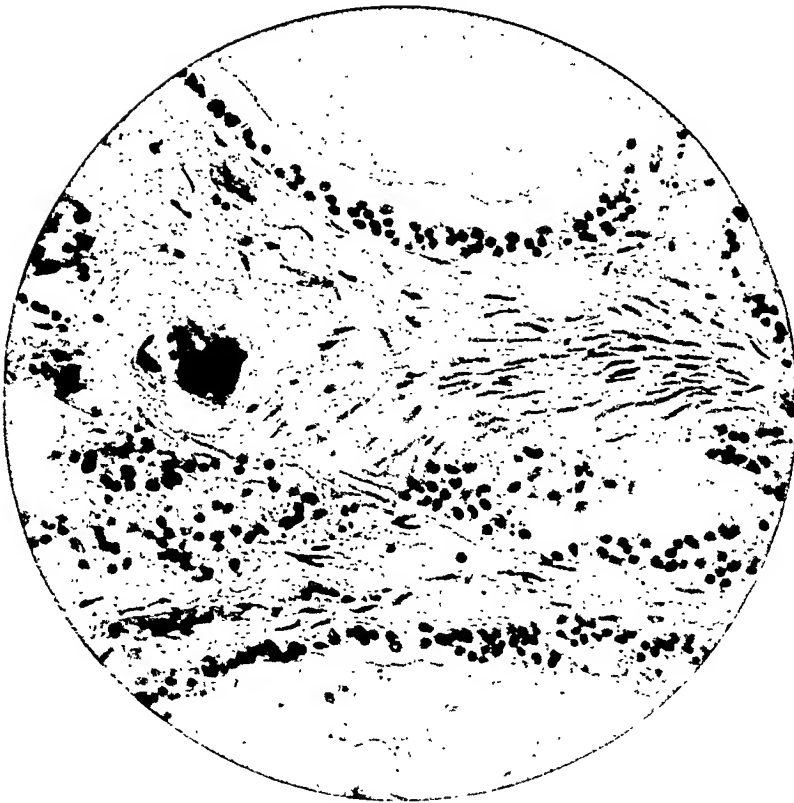
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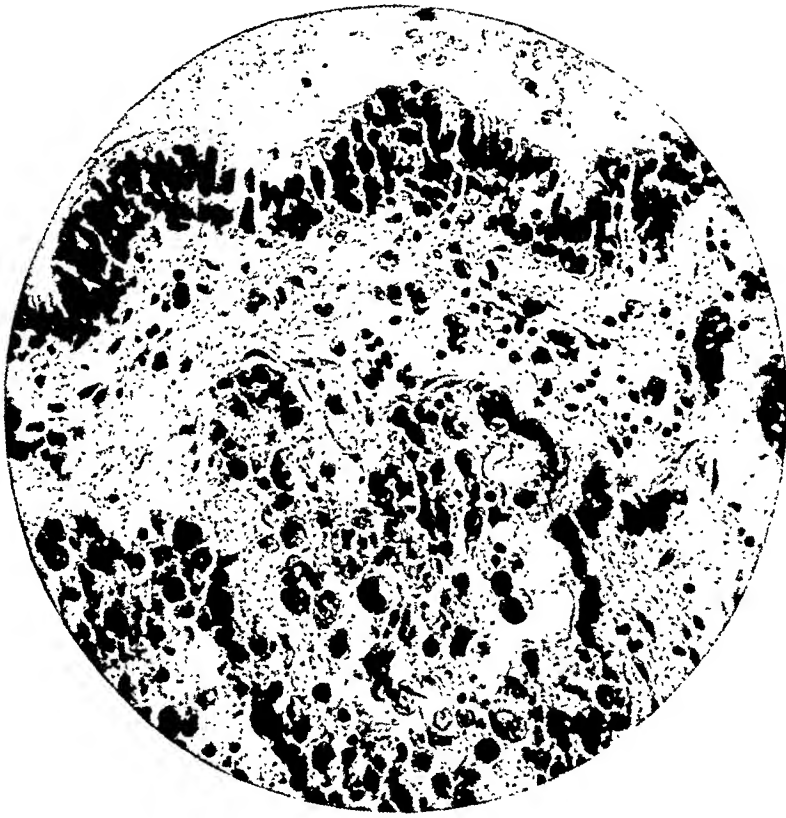
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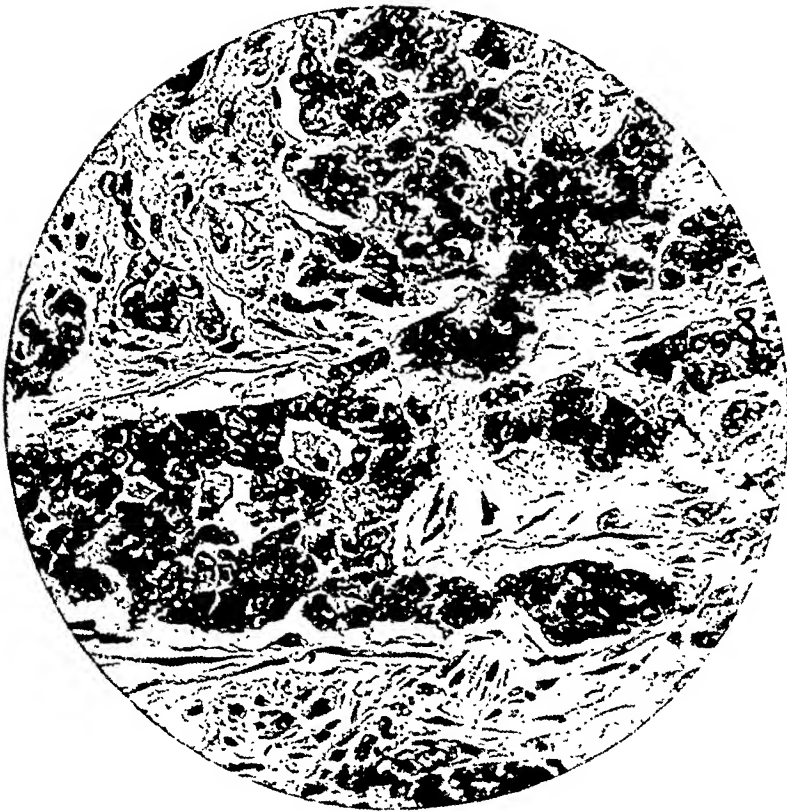
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Rusk and Epstein

Adenocarcinoma of the Uterus in a Rabbit



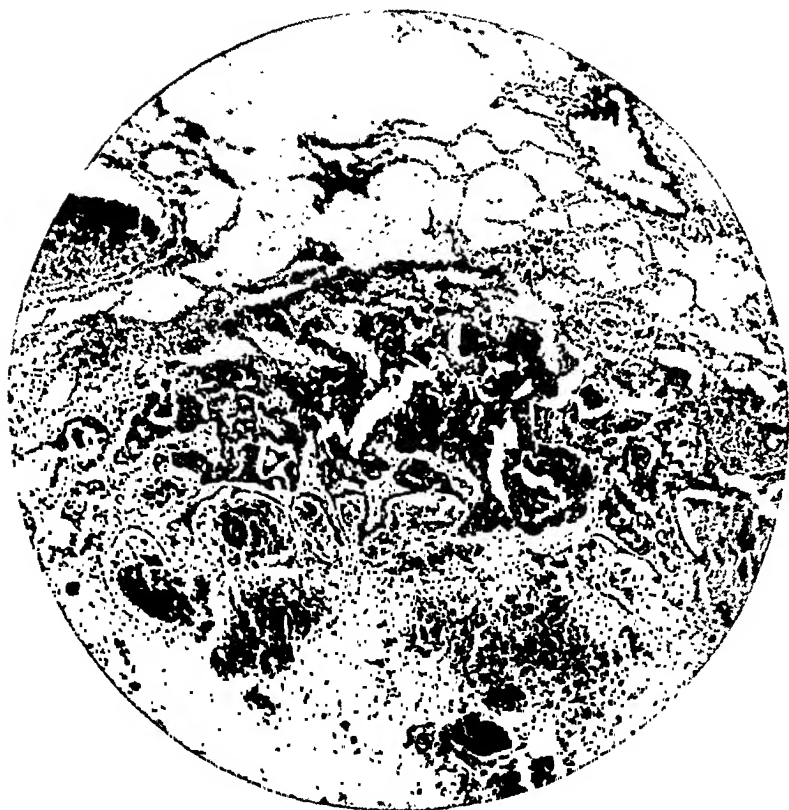
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to the development of the tongue lesions. This case is of particular interest because of the fact that the authors were able to effect a healing of the tongue lesion by repeated injections of Koch's old tuberculin.

Besley⁵ reports a case in a male age 52 with active pulmonary tuberculosis with sputum showing the bacilli. Besley's case had previously been erroneously diagnosed as tongue carcinoma.

Taddei⁶ reports a case in a woman 52 years old with a tongue lesion of two months duration. The tongue lesion was perhaps primary since there was no other evidence of the disease. The nodule ulcerated and was removed, and a guinea-pig inoculation with some of the infected tissue was successful in demonstrating the true nature of the infection.

Handfield-Jones⁷ gives a thorough presentation of the pathologic and surgical aspects of the disease, and presents five cases, three males and two females. One patient, a male, who was affected with pulmonary tuberculosis had a history of having bitten his tongue seven weeks before ulceration appeared. Three of Handfield-Jones's cases had unmistakable pulmonary tuberculosis, with the tongue lesion appearing secondary, while in two there was no evidence of primary tuberculosis elsewhere. The ages of the males were 59, 28 and 42 years respectively, while the females were 37 and 42 years respectively. The average age of this series was 41.

Fantozzi⁸ describes an interesting case in a woman age 52, who developed a tuberculous lesion of the tongue, about one month after an injury to the zygoma region which was incurred by falling to the floor.

Morrow and Miller⁹, in a splendid paper, report sixteen cases in which all but one were males. Of the sixteen, fifteen were secondary while one was perhaps primary. These authors also present a table showing that 40 per cent of the patients in their series were between the ages of 30 and 40, the average age of all patients being 41.7 years. They also mention as a possible explanation of the high incidence of the disease in the male, the fact that he is more subject to trauma of the tongue from carious teeth, pipe-smoking and the frequent practice of putting metallic objects like nails into the mouth.

The sixteen cases reported were from a clinic of 1444 tuberculous patients during a period of four years. This would give the percentage of incidence at a little less than 1 per cent.

TUBERCULOSIS OF THE TONGUE *

WITH A CASE REPORT

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The tongue appears to possess considerable immunity against invasion by the tubercle bacillus, and while tuberculosis is undoubtedly more frequently encountered in this organ than the modern textbooks would indicate, yet a review of the literature suggests that this particular manifestation of the disease is infrequently reported.

To Portal¹ is usually given the credit for describing the first case of tuberculosis of the tongue. Portal's paper was published in 1804, and since that time practically every decade has seen a sprinkling of cases appearing in the literature.

It is not the intent to present here a complete bibliographic review of tuberculosis of the tongue. To attempt this would result in a duplication of effort since such a digest is available in the excellent paper by Scott², who reviews the literature to 1916 with a collection of 231 cases, 26 of which were primary. Scott also contributes one case. The patient was a soldier aged 32 years. The lesion on the tongue existed for four years, during which time the man was repeatedly examined for pulmonary tuberculosis with negative results. It was later determined that both lungs possessed a tuberculous involvement. Since the publication of Scott's paper a number of new cases have been reported and a brief summary of these follows.

Durante³ discusses the pathologic and surgical aspect of the disease and describes five cases from material in the Mayo Clinic. Three of the cases were males aged 24, 40 and 46 years respectively, while the ages of the females were 30 and 46 years respectively.

Three of the cases were associated with the respiratory type of the disease, while in two it was not possible to determine any involvement except the tongue. Durante ends his paper with a formidable list of references on the subject.

White and Marcy⁴ describe a case in a man who had suffered from pulmonary tuberculosis for about four and one half years previous

* Received for publication January 24, 1927.

total is slightly above this figure since it is difficult for any one author to be quite sure of having reviewed all the literature on such a subject as this.

From the above chart we see that forty-three of the fifty-two cases were males while only nine were females. The age of greatest incidence is interesting in that the majority of the cases were in the early forties, with the average for the entire fifty-two cases being 42.7 years. The tongue form of this disease is rarely primary, there being but thirteen of the fifty-two cases which might be classified as such. However, as mentioned in the footnote, it is decidedly difficult to classify tongue lesions as primary, unless the possibility of tuberculous infection elsewhere is ruled out by a necropsy or other suitable procedures. Using Scott's² figures again, we find that out of the total 283 cases there are only thirty-nine which might be classed as primary. This emphasizes the importance of a thorough search for tuberculosis in some other part of the body when the infection is found in the tongue.

Morrow and Miller's⁹ statement that they found a little less than 1 per cent of a tuberculous population of 1444 affected with the tongue form of the disease, is very suggestive that tuberculosis of the tongue is considered to be rare. It might be suggested that if special attention were given to this organ during the physical examination of the living, and at necropsy of those dying of tuberculosis, the reputed rarity of this form of the disease might be disproved.

The strikingly high percentage of males over females is remarkable and has not, in my opinion, been logically explained by those who have studied and described this form of tuberculosis. Dental caries and sharp jagged teeth have been attributed as factors influencing the high incidence in the male, but without sufficient proof. I have been informed by dentists that jagged points are seen more frequently in men than in women due perhaps to chewing of tobacco which causes an uneven wearing of the teeth. The sharp points may in turn cause abrasions. However, it cannot be properly assumed that all or even the majority of men affected with tuberculosis of the tongue have been chewers of tobacco. On the other hand, it is probably correct to assume that none of the women affected was addicted to this habit. The evidence here while perhaps suggestive is not conclusive and certainly is not sufficient to explain the matter in its entirety. Some mention that men in certain vocations, such as car-

Finney and Finney¹⁰ report fifteen cases of which thirteen were males and two females. Five were thought to be primary. Three of the cases were mistaken for carcinoma of the tongue and were operated upon for this condition. The majority of these authors' cases were in the forties, the average for all being 41.7 years.

Henry¹¹ describes four cases, all males, suffering from pulmonary tuberculosis, with the tongue lesions secondary. The sputa of all four patients contained tubercle bacilli in considerable numbers. The ages of Henry's cases were 54, 29, 24 and 40 years respectively. The average for all was 37 years.

Bass¹² gives a description of the disease together with an extensive bibliography, and presents two cases, one a physician age 43 years, male, with a doubtful history of tuberculosis elsewhere; the other a

TABLE I
Summary of the Cases Reported above

Author	Number of cases	Sex		Age average	Lesion	
		Male	Female		Primary*	Secondary
Scott ²	1	1	0	32	0	1
Durante ³	5	3	2	37	2	3
White & Marcy ⁴	1	1	0	30	0	1
Besley ⁵	1	1	0	52	0	1
Taddei ⁶	1	0	1	52	1	0
Handfield-Jones ⁷	5	3	2	41	2	3
Fantozzi ⁸	1	0	1	52	1	0
Morrow & Miller ⁹	16	15	1	41.7	1	15
Finney & Finney ¹⁰	15	13	2	41.7	5	10
Henry ¹¹	4	4	0	37	0	4
Bass ¹²	2	2	0	54	1	1

* The word primary is used here in a restricted sense, meaning that in most instances it was impossible, from the available information to determine primary lesions elsewhere.

male age 66 years having in addition to the tongue lesion a tuberculous adenitis. This second case possessed no history or clinical evidence of tuberculosis except the tongue and submaxillary lymph nodes, although previously, two wives of this man had succumbed to the disease.

The fifty-two cases reported above added to the 231 cases previously collected by Scott² give a total of 283 cases of tuberculosis of the tongue which have been reported to date. Perhaps the actual

of tongue tuberculosis, or at the most the influence of this habit must be negligible.

It is likewise difficult to account for the age incidence. The ages of the majority of the reported cases have been in the early forties. This, together with the fact that most of the tongue lesions have been secondary to a pulmonary involvement, would suggest that the tongue is more prone to attack in the later stages of the disease and at a time when the resistance may be diminished.

REPORT OF A CASE

The patient, auto salesman, age 36, in the practice of Dr. W. A. Kickland, had suffered with pulmonary tuberculosis since he was 22 years of age (fourteen years). Four months before the tongue lesion developed he broke a tooth and bit his tongue with the remaining sharp fragment. A painful nodule appeared in the same area, which was on the right lateral surface, about one inch from the tip. Soon after the appearance of the nodule, another physician removed a portion, and reported the presence of acid-fast bacilli. The lesion was then cauterized with silver nitrate. The nodule persisted however, and two months later the lesion was removed surgically and the wound electrically cauterized. The area has been treated at intervals with the ultraviolet ray, and at the present time the patient appears to be making a satisfactory recovery, although it is probably too early to venture an ultimate prognosis.

Pathology. Sections were prepared from the tissue which was removed at the operation, and stained with hematoxylin and eosin. Others were stained with hematoxylin and Ziehl-Neelson's carbol fuchsin for the purpose of demonstrating any acid-fast organisms present. Sections were cut at different planes to facilitate a comprehensive study of the material.

Immediately beneath the mucosa there was a large irregular area of early ulceration or necrosis quite devoid of giant cells, and with no evident attempt at encapsulation (Fig. 1). Adjacent to this zone the muscle for a considerable depth was involved in a typical tuberculous process. The lesion consisted of tubercles which occupied the muscle bundles or fascicles and were rather sharply separated from each other by the remains of the perimysium (Figs. 2 and 3). Many of the muscle bundles contained only a remnant of muscle fibers which

penry and shoe repairing, frequently carry in their mouths metallic objects such as nails, and, of course, the likelihood of trauma that would permit the entrance of the tubercle bacillus into the mucosa and underlying tissues is considerable. This explanation of the high incidence of tuberculosis of the tongue in the male is entirely inadequate when one considers that carpenters and shoemakers were conspicuous by their absence in the case histories reported.

While I feel that the majority of the tongue lesions arise from the inoculation of a break in the continuity of the mucosa of the organ, usually in the nature of a traumatism, I fail to recognize any factor of a material kind that sufficiently accounts for the predominance of cases in the male.

In a matter of this kind, one is tempted to suggest as a hypothesis a sex susceptibility which may be possessed by certain males, and to a lesser degree or not at all by females. The apparent natural immunity of striated muscle to the tubercle bacillus has been commented upon many times, and is perhaps the explanation in part at least of why the tongue is so rarely involved. The proposition of susceptibility is of sufficient importance to be considered at least a contributory factor in those in which the tongue is affected. The influence of susceptibility and immunity must remain a matter of conjecture since adequate proof at present is impossible to assemble.

There can be no doubt that the tongue of all sufferers of open pulmonary, pharyngeal and laryngeal tuberculosis is continuously exposed to secondary infection by the bacilli laden secretions with which the tongue is in constant contact. The wonder is that it succumbs so infrequently to invasion.

In a few instances the cases possessed a history of tongue lacerations by self-inflicted bites. Such trauma is common even in the non-tuberculous as the personal experience of all of us could testify. No doubt many bite wounds that later became sites of tuberculous lesions were considered inconsequential and promptly forgotten. As a consequence perhaps bite wounds are not mentioned as frequently as their true position in the pathogenesis of the disease warrants.

Some have placed, I think, undue emphasis upon the effect of pipe-smoking as a contributory factor in causing the tongue lesion. From the evidence presented in the reported cases, it is difficult to look upon pipe-smoking as playing any rôle whatever in the pathogenesis

the use of the cautery at the time of the operation and the subsequent employment of the violet ray were considered proper procedures.

SUMMARY

1. A review of the literature available since 1916, dealing with tuberculosis of the tongue is presented.

2. About fifty-two cases have been reported during the past ten years. These added to those previously reported bring the total of cases up to 283.

3. The assembled data indicate that the disease is nearly five times as frequent in the male as in the female.

4. The average age incidence of the fifty-two cases reviewed is 42.7 years.

5. An original case is described.

The writer is indebted to Dr. W. A. Kickland, who kindly furnished the information necessary for the history of the case reported.

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were badly distorted due to the pressure exerted by the adjacent tubercles. The intermediate areas of some of the tubercles showed considerable accumulations of endothelial leucocytes with an occasional lymphocyte. In the extreme depths of the tissue a few muscle bundles had been replaced by collections of epithelioid (endothelial) cells without giant cell formation. These accumulations appeared to be very early tubercles, many of which were separated from the great zone of tubercles by bundles of normal muscle fibers indicating that perhaps the mode of extension of the infection was at least in part hematogeneous. The tubercles appeared to be of the same duration and fairly young as none showed caseation or calcification. A few of the tubercles showed only one giant cell, but the majority possessed two or three, and many five to seven (Fig. 3). Blood channels in the affected portion were few, and the perivascular infiltration noted by others was not observed.

Acid-fast bacteria of the morphology typical of the tubercle bacillus were demonstrated in a few of the sections stained with hematoxylin and carbol fuchsin. The organisms were located within the body of the giant cells and were very few in number.

DISCUSSION

From the history of the above case together with the pathologic findings there can exist no reasonable doubt that we were dealing with a case of tuberculosis of the tongue. The pathology also bears out the history that the lesion was of recent origin. The multiplicity of the tubercles, the majority of which were apparently of the same age, would indicate a rather massive inoculation, and a general susceptibility of the muscular fibers to the infection. While the original infection of the tongue undoubtedly resulted as a direct inoculation of the bite wound by the bacilli, there is some reason to think that part of the subsequent extension has been by way of the local blood vessels. It would be difficult to account for the young tubercle formations in the depth of the tissue by any other manner. The extension in the deeper portions by continuity would appear to be quite improbable.

The multiplicity of the tubercles and their widespread distribution even up to the edge of the line of incision, would cause one to doubt the likelihood of all the diseased tissue being removed. Consequently

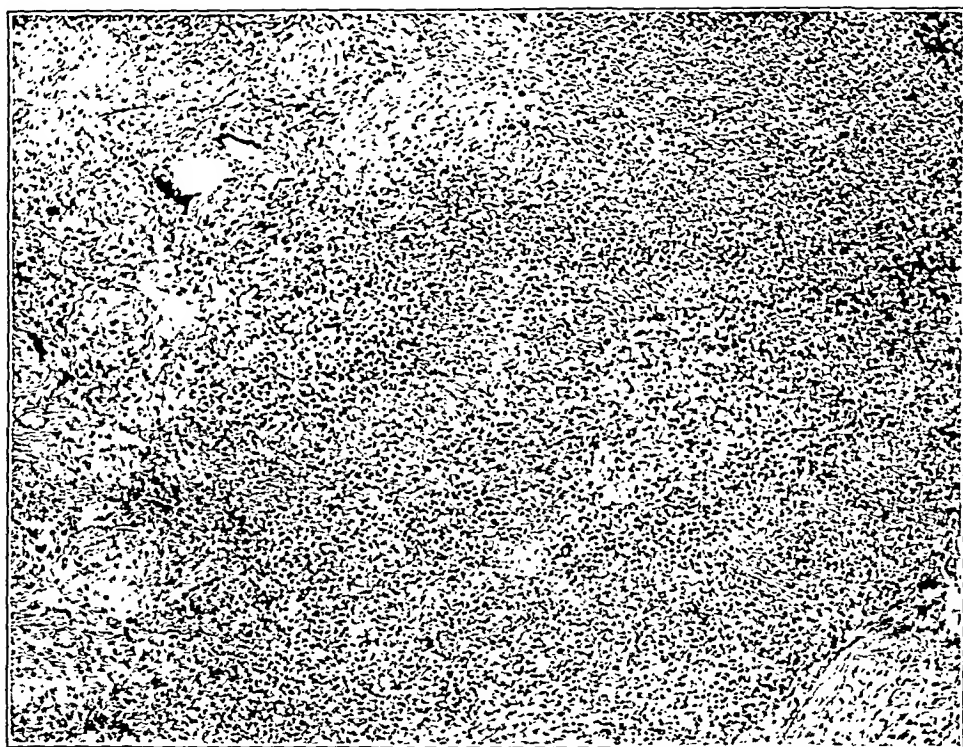
DESCRIPTION OF PLATES

PLATE 78

- FIG. 1. Tuberculosis of the Tongue. Low power view of an area in the sub-mucosa showing ulceration.
- FIG. 2. Tuberculosis of the tongue. Low power photomicrograph of a tubercle occupying the space normally occupied by a bundle of muscle fibres. Note the many giant cells and the sharp separation of the tubercle from the surrounding tissue.

PLATE 79

- FIG. 3. Tuberculosis of the tongue. Low power photomicrograph of a tubercle showing a large number of typical giant cells.
- FIG. 4. Tuberculosis of the tongue. High power photomicrograph of a giant cell.



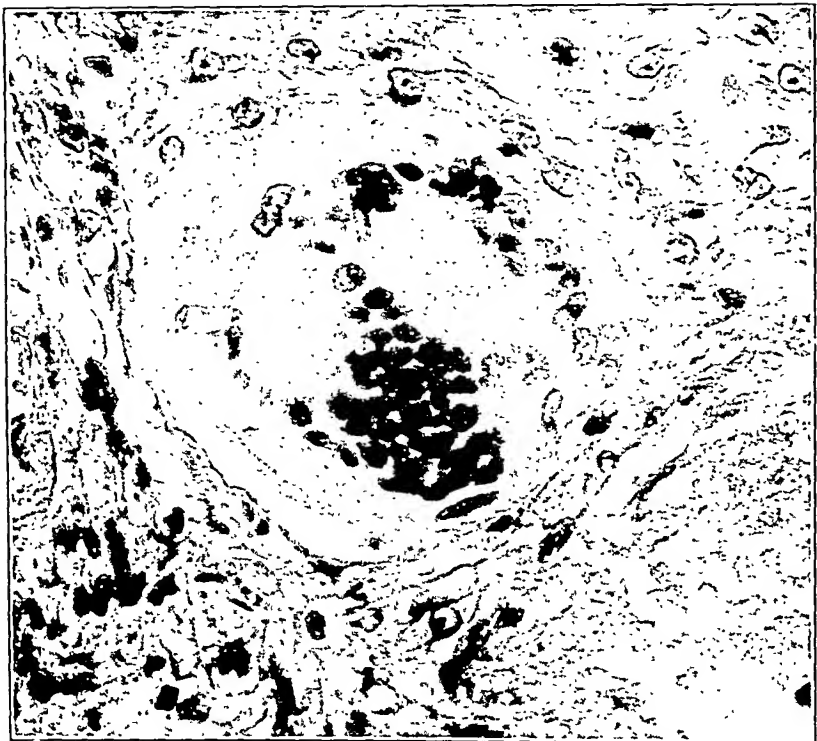
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experiments approached therefore homoiotransplantations.* Each guinea-pig was checked by a number assigned serially and given here in brackets. Each inbred mating is designated by three figures. The first is the family number (2, 13, 32, 35 and 39); the second is the number of generations of brother-sister mating since the foundation (O) mating of the family; while the third is arbitrary. Experiments 2N, Y and 35I involve only families 2, 13 and 35 respectively but are not brother-sister matings. Experiment CO is composed of crosses between inbred families.

I. TRANSPLANTATIONS IN THE SAME FAMILY, HOST AND DONOR NOT BEING NEARLY RELATED

SERIES A. *Experiments in which the examination took place within two months following transplantation.* Weight of guinea-pigs in most cases varying between 200 and 500 gm.

1. From guinea-pig 32-17-10 (15611) to guinea-pig 32-19-8 (321), 40 days. Both thyroid and cartilage transplants are like perfect autotransplants, as far as structure and lack of increase in lymphocytes and connective tissue are concerned. Grade 6.

2. From 2-17-10 (15959) to 2-17-5 (15694) 50 days. Thyroid, parathyroid, and cartilage autotransplants. Grade 6.

3. Y-4 $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-5 \end{array} \right\}$ (16039) to Y-7 $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-6 \end{array} \right\}$ (16029) 40 days.

Syngenesio-reaction. Grade 5. Thyroid shows auto-structure, but there is much lymphocytic infiltration in certain areas of the center. From here lymphocytes penetrate between acini toward periphery and surround some acini. Around a vessel traversing the ring of acini are lymphocytes. In other places the center is free from lymphocytes. Cartilage transplant with areolar tissue is well preserved; but there is some increase of connective tissue around the cartilage and an incomplete mantle of lymphocytes.

4. From 32-17-11 (15575) to 32-17-8 (15634) 40 days. Thyroid autotransplant. Grade 6.

5. From 13-19-9 (15649) to 13-20-5 (15563) 35 days. Syngenesio-reaction. Grade 5. Thyroid with auto-structure; parathyroid well preserved, but intense lymphocytic infiltration around and in parathyroid, and in places in the periphery of the thyroid. In various

* These figures have been mentioned in a previous paper.

TRANSPLANTATION AND INDIVIDUALITY DIFFERENTIALS IN INBRED FAMILIES OF GUINEA-PIGS *

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In a series of papers one of us analyzed the reactions of the host against auto, syngenesio, homoio and heterotransplants.¹ Definite relations were found between the intensity of the lymphocytic and connective tissue reactions and the genetic relationship between host and donor. The similarity or differences between the individuality differentials of host and transplant largely determined the effects of the transplantation. These relations between individuality differentials decided whether the substances given off by the transplant affected the host as normal auto substances or as toxins of various intensities (syngenesio, homoio, heterotoxins). In the case of auto-transplantation, there exists an identity of individuality differentials. The intensity of the reactions appearing after transplantation furnished a quantitative measure of the similarity or difference between individuality differentials. It was to be assumed that through long-continued inbreeding the individuality differentials among the members of the inbred family would gradually become more and more alike. In addition to these primary factors affecting the individuality differential there were active secondary factors of a non-genetic character.

Under these conditions it was of interest to extend these experiments to inbred strains, and this paper deals with the results obtained in the exchange of tissues in families of guinea-pigs which have been inbred in the United States Department of Agriculture since 1906. Five different families have thus been developed through continued sister and brother matings (2, 13, 32, 35 and 39). However, the degree of homogeneity in the various families differs.² In addition, control experiments were made in which tissues were exchanged with members of a B group. In this group, originally derived from the same stock as the inbred families, matings as close as those between second cousins have been avoided. These latter

* Received for publication February 25, 1927.

SERIES B. *In a second similar series the examination took place at a still later date in the majority of cases.* In these experiments the weight of the animals was generally greater, in accordance with the greater age of these guinea-pigs.

1. From 13-20-5 (15563) to 13-20-13 (15782) 5 months, 12 days. Transplanted thyroid was not found.

2. From Y-7 $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-6 \end{array} \right\}$ (16029) to 13-20-13 (15782) 5 months, 12 days. In this case there is a decided syngenesio-reaction. A part of the transplanted thyroid was preserved and the acini contain well formed colloid. But there is much lymphocytic infiltration. The lymphocytes penetrate everywhere between the acini and even into the epithelium and destroy it. There is also some increase of fibrillar connective tissue around some acini. Lymph vessels are filled with lymphocytes. Large masses of these cells surround and encompass acini which have lost their colloid. Grade 4.

3. From 2N-27 (18040) to 2-17-14 (18450) 102 days. Thyroid and cartilage were perfect autotransplants. Grade 6.

4. From 13-19-15 (18317) to 13-21-7 (18493) 102 days. Thyroid, parathyroid and cartilage with surrounding tissue resemble autotransplants, except that in one place in the cartilage transplant there was a slight collection of lymphocytes around a vessel; this latter condition may still come within the range of autotransplant. Grade 6 or 5.75.

5. From 2-16-16 (15590) to 2N-26 (15673) 104 days. Auto-reactions in thyroid and cartilage transplant. Grade 6.

6. From 32-19-8 (15634) and 32-19-8 (15633) to 32-19-9 (16900) 37 days. Two thyroids, and parathyroids transplanted. Auto-reaction, except that in one place in the center there is a considerable lymphocytic infiltration. Grade 5.60.

The six experiments of this series confirm and extend the conclusions of the first series. Experiment 1 is uncertain as to the interpretation of the results, no thyroid transplant having been found after 5 months, 12 days. It is possible that the transplant has been destroyed after such a long period, especially considering the fact that transplantation was carried out in family 13, in which there is apparently a greater tendency on the part of the host to react against the transplant from another individual of the same family. A marked reaction was obtained in family 13 in the second case follow-

places small parts of thyroid have been destroyed by lymphocytes which accumulate around vessels. Normal cartilage surrounded by areolar and fat tissue, with only a slight increase in connective tissue and lymphocytes.

6. From 13-20-5 (15563) to 13-20-13 (15782) 31 *days*. Syngenesio-reaction. Grade 5. Lobes of thyroid were transplanted (parts of the transplants were used for retransplantation). Structure of autotransplants, but in center there is moderate, although distinct, lymphocytic infiltration; lymphocytes penetrate also between acini and surround and destroy some of them. Moderate increase of connective tissue between acini.

7. From 32-18-9 (20865) to 32-19-9 (16900) 38 *days*. Resembles autotransplant. Grade 6.

8. From 2-18-4 (15693) to 2-16-16 (15590) 49 *days*. Syngenesio-reaction. Grade 5. Well preserved thyroid. Considerable lymphocytic infiltration penetrating a little toward periphery and also into parathyroid. Slight connective tissue increase around cartilage. Slight increase of lymphocytes in areolar tissue.

Eight experiments constitute this series. In four cases an auto-reaction was obtained; in four cases a syngenesio-reaction. In the latter cases the transplant first began to develop as an autotransplant, but later, lymphocytes began to invade the transplant and even connective tissue penetrated between some of the acini. We have, therefore, in these cases to deal with syngenesio-reaction. Auto-reactions were found 38, 40 and 50 days after transplantation, syngenesio-reactions 30-50 days after transplantation. Auto-reactions were obtained in families 32 and 2, syngenesio-reaction in family 13, and in one case in family 2. There can be no doubt that a difference existed in the constitution of the individuality differentials in the members of families 13 and 2 which showed syngenesio-reaction. In the two cases in family 32 and in one case in family 2 the reactions did not indicate a difference in individuality differentials, but this does not necessarily exclude the possibility that such a discrepancy would not have been revealed if we had extended the experiments over a still longer period. On the other hand we can state definitely that the members of the same inbred family are more nearly akin to each other as far as the constitution of their individuality differential is concerned, than in ordinary guinea-pig families where brothers are related to each other.

also parathyroid and surround some acini of thyroid. Cartilage similar to autotransplant; but there is in places in the surrounding areolar and fat tissue a slight increase in connective tissue with some lymphocytes around vessels. Near such places in fat tissue are found some giant and epithelioid cells; elsewhere fat tissue is free from the latter structures. Grade 5.

2. From C-O-285 $\left\{ \begin{array}{l} 35-21-2 \\ 32-15-15 \end{array} \right\}$ (15942) to
C-O-240 $\left\{ \begin{array}{l} 32-16-9 \\ 35-16-11 \end{array} \right\}$ (15943)

35 days. Thyroid and cartilage resemble autotransplant. Grade 6. The number of experiments in this series is too small to allow of any definite conclusion.

SERIES D. *Successive transplantation in the same family.* In a few cases, pieces were transplanted to an animal of the same strain, and after these pieces had been in the first host for some time, they were removed and retransplanted into a second host belonging likewise to the same strain. After a certain period these retransplanted pieces were taken out and examined microscopically.

1. First transplantation. From 13-20-5 (15563) and from

$$Y-7 \left\{ \begin{array}{l} 13-20-2 \\ 13-19-6 \end{array} \right\} (16029) \text{ to } 13-20-13 (15782) \text{ 31 days.}$$

Second transplantation. 13-20-13 (15782) to 13-18-9 (16174) 23 days. Thyroid shows structure of autotransplant: a ring of acini with a small amount of connective tissue in center. The majority of acini contain colloid, others do not. Between acini there is fibrillar connective tissue with much lymphocytic infiltration; a number of acini have been destroyed by lymphocytes or connective tissue. The lymphocytic infiltration is in places moderate and in others more marked, though not overwhelming. Lymphocytes invade some acini. Grade 3.75. Here in family 13 is a very decided syngenesio-reaction. The piece was in a strange host during a period of 54 days. The marked reaction found in this case is in accordance with the fact that the second host, 16174 (13-18-9) had only 7 generations of brother-sister ancestry in common with either of the donors.

2. First transplantation. Cartilage transplanted from guinea-pig 13-21-19 (20898) to 13-20-17 (20761) 6 days.

Second transplantation, 13-20-17 to 13-23-1 (20986) 7 days. Duration of experiment 13 days altogether. The original cartilage

ing a transplantation extending over a long period of time. In the fourth case, on the other hand, we find after almost three and a half months a condition approaching that found in autotransplants. It is, of course, to be assumed that in certain combinations of animals of this family the individuality differentials may happen to be identical or almost identical. In two experiments in family 2 the results indicated an identity, or a condition almost approaching identity, of the individuality differentials. In one experiment in family 32, extending over 37 days, the result at present is similar to that obtained in autotransplantation, but there is some indication that with an extension of the experiment, the differences between the individuality differentials would come out definitely. These experiments then demonstrate the lack of identity of individuality differentials in family 13 and probably also in family 32. The pedigree analysis indicates that the large number of reactions not auto in family 13 is probably due to the large number of experiments in which host and donor had less than 10 generations of common inbreeding. At the same time these experiments prove that the similarity of individuality differentials is still greater than appeared in the first experiment, inasmuch as even after relatively long periods of time the conditions obtained may still correspond to those found in autotransplantation. And even where, as in some cases in family 13, a definite discrepancy between the individuality differentials has been found, the latter are still much more closely related than the average of the individuality differentials of brothers in non-inbred families of guinea-pigs.

These experiments demonstrate anew the great sensitiveness of the lymphocytic reaction which may appear at a very late stage of transplantation and then exert a destructive effect in case the synergistic toxins are very weak. There may be also associated with this lymphocytic reaction a secondary slight proliferation of the connective tissue in the transplant.

SERIES C. *Transplantation of tissues from a hybrid between two inbred species to another hybrid of similar constitution.*

i. From C-O-297 $\left\{ \begin{smallmatrix} 2-17-8 \\ 35-23-10 \end{smallmatrix} \right\}$ (17093) (320 gm.) to
C-O-234 $\left\{ \begin{smallmatrix} 2-13-7 \\ 35-16-20 \end{smallmatrix} \right\}$ (15880) (632-682 gm.)

31 days. Thyroid with auto-structure; in one place in center there is a considerable lymphocytic infiltration; the lymphocytes infiltrate

37 days. Two thyroids examined. Both lobes behave like or almost like autotransplants, but there are collections of lymphocytes in lymph vessels and in various places in the fibrous tissue around the thyroid transplant. Grade 5.60.

2. Liver, spleen and pancreas from 13-19-9 (15648) to 13-18-10 (16787) 36 days. The spleen shows lymph follicles in its periphery, pulp with blood sinuses and strands of hyaline connective tissue probably corresponding to trabeculae. Perhaps in the periphery of the capsule there is some lymphocytic infiltration. The lymph follicles have larger cells in their centers and smaller cells in the periphery. In the liver, the bile ducts are preserved and are actively proliferating, showing mitoses. They are surrounded by a fibrous capsule with slight lymphocytic infiltration. The liver tissue is well preserved, and apparent transitions between bile ducts and liver tissue are visible. Mitoses seem to occur in the liver cells. There are small collections of lymphocytes here and there in the liver tissue. The pancreas shows only fat tissue with an epitheloid, giant cell and lymphocytic infiltration. Grade about 5.50.

3. Adrenals from Y-4 $\left\{ \begin{array}{l} 13-20-2 \\ 13-19-15 \end{array} \right\}$ (16038) to 13-18-10 (16788) 36 days. Normal adrenal tissue. There are cells containing yellow pigment; and fat cells are also present. In places, cholesterol crystals are surrounded by foreign body giant cells. There is fibrillar connective tissue in the periphery. No lymphocytic infiltration. Grade 6.

4. Two thyroids from 13-18-10 (16787) and one spleen from 13-21-11 (21161) to 13-21-19 (20897) 27 days. Thyroids and parathyroids closely resemble autotransplants, showing very little lymphocytic infiltration in the center. Spleen contains Malpighian bodies with large endothelial cells in their centers. There are mitoses in and around lymph follicles; pulp with blood vessels and some connective tissue; mitoses in the endothelium of the sinuses; hemorrhage, and in the periphery of the transplant, collection of lymphocytes. Grade 5.25.

These experiments prove again that in family 13 a complete homogeneity of individuality differentials has not yet been attained. As in our previous experiments the individuality differentials resemble each other more on the average than the individuality differentials of brothers in non-inbred families. We find that spleen, adrenal, and liver tissue can be successfully transplanted in such

though showing areas of necrosis, also shows some newly-formed perichondrial cartilage. The fat tissue which is preserved is invaded in places by connective tissue. There is much fibrillar connective tissue around the cartilage. In the absence of a lymphocytic infiltration it is doubtful whether the connective tissue increase is to be considered as a specific reaction against the transplant.

3. First transplantation. Four thyroids from two guinea-pigs 32-19-8 (15634 and 15633) to guinea-pig 32-19-9 (16900) 37 days. The specimen is like an autotransplant or nearly so, with perhaps a slight increase in connective tissue.

Second transplantation, 32-19-9 (16900) to brother 32-19-9 (16899) 4 months, 9 days. Thyroid is like an autotransplant with low to medium-sized epithelium of acini which are close together and contain solid, much retracted colloid. There is a small amount of fibrous tissue in the center. The transplant is surrounded by fibrous tissue. The parathyroid is negative, although in the capsule around the parathyroid there are some lymphocytes. Grade 6 or 5.75. In this case the second transplantation was made into the brother of the first host. The total duration of experiment 2 was five and one-half months, and yet no definite reaction against the tissue has occurred. This demonstrates the great similarity of individuality differentials in this case. These experiments further demonstrate the feasibility of serial transplantations with as sensitive an organ as the thyroid gland, provided the individuality differentials of the hosts are similar to those of the donors.

SERIES E. *Multiple simultaneous transplantations in the same family, with examination within 6 weeks after transplantation.* In this and in the following series the variety of organs which were transplanted was enlarged; in addition to thyroid, parathyroid, and cartilage, such organs as spleen, adrenal, liver, pancreas, and bone, were also transplanted. In the first experiments four thyroids from two different donors were transplanted. It was of interest to test how more sensitive pieces of organs or tissues would behave under the favorable conditions in which the individuality differentials in host and donor are so similar to each other. Five experiments were made in this series.

- I. 32-19-8 (15634) and 32-19-8 (15633) four thyroid lobes to 32-19-9 (16900)

an intense lymphocytic infiltration is beginning in other places, the appearance is that of a lymph gland and the thyroid is being destroyed. The lymphocytes infiltrate also the interstices between acini and later enter the acini. Apparently some remnants of liver in the form of yellow tissue; much fibrous tissue formation and dense masses of lymphocytes. Lymph vessels filled with lymphocytes. Grade 4. This case illustrates the secondary late infiltration and destruction of syngenesiotransplants by lymphocytes.

6. Thyroid, cartilage, adrenal and liver from 2-17-30 (23458) to 2-19-13 (23347) 132 days. No thyroid or liver found. Cartilage well preserved, but some lymphocytic collections around cartilage and in fat tissue; they are larger than they would be in autotransplants. Well preserved muscle tissue, probably transplanted. Tissue consisting of yellow vacuolar cells with nuclei, surrounded by fibrous tissue with lymphocytic masses. Tissue largely infiltrated and destroyed by lymphocytes. (Adrenal?) Grade 4.75?

7. Cartilage, ovary, liver, adrenal from 32-23-2 (23420) to 32-18-7 (21067) 129 days. Well preserved cartilage and bone, surrounded by fat tissue. No increase in connective tissue, no lymphocytes. Proliferating zone of cartilage cells near bone. Bone marrow preserved. Cartilage partly vacuolar and dissolved; no lymphocytes. Ovary; preserved follicles of all sizes and normal ova, follicles in stage of granular degeneration, some atretic follicles. Germ layer: fibrillar connective tissue and muscle tissue well preserved. Medullary ducts; almost normal ovary. Liver and adrenal not found. Grade 6.

8. Cartilage, ovary, spleen from 2N-31 (19998) to 2-17-23 (23428) 132 days. Cartilage well preserved surrounded by fibrillar connective tissue. Distinct lymphocytic infiltration around cartilage. Over wide areas no lymphocytic infiltration. Two ovaries with much fibrous tissue and dense lymphocytic infiltration; also fimbria epithelium with lymphocytic infiltration. Atretic follicles with zonae pellucidae. One of the two ovaries has no good follicles, the other has primordial follicles with normal ova, small follicles partly without ova, and medullary ducts, the epithelium of which has been penetrated by lymphocytes. Spleen consists of fibrous tissue with hemorrhage. Connective tissue grows into transplant. Apparently some reticulo-endothelial tissue left. There is lymphocytic infiltration. It is difficult to determine how much of these lymphocytic

inbred families. Especially in the liver transplant, mitoses are found in bile ducts as well as in liver cells as late as 36 days after transplantation.

SERIES F. Multiple simultaneous transplantations in the same family, examination taking place more than four months after transplantation.

1. Thyroid, cartilage, bone, liver and adrenal from 32-20-9 (23396) to 32-20-7 (23588) 129 days. Thyroid, parathyroid, cartilage, bone marrow with megalokaryocytes preserved; bone and proliferating zone of cartilage near bone well preserved. Liver not definitely found. Grade 6.

2. Thyroid, cartilage, liver and adrenal from 13-23-3 (23525) to 13-21-13 (23448) 132 days. Thyroid, parathyroid, cartilage, bone, with zone of proliferating cartilage cells, bone marrow, with myelocytes, leucocytes, megalokaryocytes, well preserved; perhaps some slight connective tissue increase in bone marrow. No adrenal or liver found. Grade 6.

3. Thyroid, cartilage, adrenal, and liver from guinea-pig 13-23-5 (23525) to 13-21-3 (23660) 132 days. Thyroid: in center a considerable mass of lymphocytes. Some lymphocytes penetrate between acini. Also in fat tissue, a mass of lymphocytes. Connective tissue in center and elsewhere increased. Good ring of acini with good colloid. Cartilage resembles autotransplant; bone marrow well preserved. No definite liver or adrenal tissue found. Grade 4.75?

4. Thyroid, cartilage and adrenal from 32-20-9 (23396) to 32-19-6 (23601) 129 days. Thyroid: lymphocytic infiltration in center and fat tissue, which extends also between acini: parathyroid markedly infiltrated and destroyed by lymphocytes. Around cartilage perhaps very slight increase in fibrous tissue and a few lymphocytes. Proliferation zone and cartilage cells near bone preserved. Bone marrow preserved, (megalokaryocytes, leucocytes) but much lymphocytic infiltration and connective tissue increase in peripheral parts. No adrenal found. Grade 5.

5. Thyroid, adrenal and liver from 2-17-30 (23458) to 2-17-18 (23551) 132 days. Thyroid: acini close together with colloid; in addition numerous other acini without colloid and some of these compressed. Over large areas very small acini with colloid staining red with eosin. The acinus cells of the small acini may be destroyed. In many places the thyroid appears still like an autotransplant; but

II. HOMOIOTRANSPLANTATIONS AS CONTROL EXPERIMENTS

Three kinds of control experiments were carried out.

A. *Transplantations from one inbred family to another inbred family.*

B. *Transplantations in control stock B.* These are guinea-pigs which originally are from the same stock from which the inbred families were derived, but in this B stock, matings as close as those between second cousins have been avoided.

C. *Transplantations from B stock to a totally unrelated stock obtained in St. Louis.* It will be possible to report on those control experiments very briefly because they behave like typical homoio-transplantations, on which one of us has reported previously on several occasions.

SERIES A. *Exchange of tissue between two inbred families.*

1. From family 32 to family 2. 20 days. Thyroid in part preserved, in part infiltrated and destroyed by connective tissue and lymphocytes. In dense fibrous tissue much lymphocytic infiltration. Also parathyroid infiltrated by lymphocytes. Cartilage: part of fat tissue replaced by fibrous tissue; lymphocytic infiltration in fat tissue and around cartilage. Grade 3.25.

2. From family 32 to family 13. 20 days. Thyroid: only fibrous tissue. Cartilage: partly necrotic; surrounded by fibrous tissue. Lymphocytes around cartilage. Bone marrow replaced by fibrillar connective tissue. Grade 1.

3. From family 32 to family 2. 20 days. Thyroid: intense lymphocytic infiltration, some small areas of thyroid preserved. Cartilage with some perichondrial regeneration. Intense lymphocytic infiltration and connective tissue around cartilage; only slight amounts of fat tissue preserved. Grade 2.5.

4. From family 32 to family 35. 21 days. Thyroid: some compressed acini without colloid, a few acini with colloid. Fibrous tissue and much lymphocytic infiltration between acini. Fibrous tissue around cartilage; fat tissue with thickened septa. Distinct lymphocytic infiltration. Grade 2.75.

5. From family 32 to family 13. 21 days. Thyroid not found. Cartilage with lymphocytic infiltration. Bone marrow transformed into myxoid connective tissue. Grade 1.5.

masses are Malpighian bodies of the transplanted spleen and how much represents lymphocytic infiltration on the part of the host. Grade 4? It is interesting to compare experiments 7 and 8. In experiment 7, the transplants behaved like autotransplants and the ovaries are in excellent condition. In experiment 8, there is decided syngenesio-reaction with lymphocytic infiltration, affecting also the ovaries; here the condition of the ovaries is much inferior.

9. Cartilage, spleen, testicle, pancreas from 13-22-11 (23543) to 13-21-13 (20808) 132 days. Cartilage normal, surrounded by fat tissue; in places small collections of lymphocytes around vessels. Very small collections of lymphocytes next to perichondrium. Testicle; tubules surrounded by much lymphocytic infiltration; no spermatozoa. Sertoli cells preserved. Spleen; sinuses separated by connective tissue; lymph follicles with large endothelial cells, some showing mitoses. Hemorrhagic areas into which connective tissue grows. Several serous cysts with much lymphocytic infiltration. Grade 5.

In three of these nine experiments the grade was 6; namely in families 32 in two cases, in family 13 in one case. In six experiments the results corresponded to syngenesiotransplantation. Grade 5, in family 32 and 13. Grade 4.75 in family 2 and 13. Grade 4 in two transplantations in family 2.

In families 32, 2 and 13 there was therefore non-identity of individuality differentials. The correspondence in the behavior of different organs in the same experiment is of interest; our previous results are thus confirmed. Of interest also is the late invasion of these transplants by lymphocytes. The invasion can become overwhelming and in the end destroy the transplant. Thyroid, cartilage, bone, bone marrow, parathyroid and ovary can relatively easily be transplanted; somewhat less readily spleen. Liver and adrenals were usually not found four months or later following transplantation. While thus individuality differentials are not yet identical in these families, they have reached a stage where they have become very similar to each other within the same family.

4. B to Bu. 40 days. Grade 1.

Average grade 1.31.

Comment. The differences between these three series of homoiotransplantation and the six series in which transplantations were carried out in the same family are very striking; in the former the total average grade is 1.6; in the latter series the average grade is 5.28. Of course, these figures can only claim to be approximate. We may state that the average grade in the transplantations within the same family is between 5 and 6, but nearer 5 than 6. There is an indication that families 32 and 13 are more strange to each other than families 32 and 2 or 32 and 35. However, this point needs further investigation. Of interest is also the marked reaction against tissues of hybrids in homoiotransplantation.

III. TRANSPLANTATION FROM BROTHER TO BROTHER WITHIN AN INBRED FAMILY

When, in an ordinary non-inbred strain, transplantations from brother to brother are carried out, the results are better than in ordinary homoiotransplantation, or in syngenesiotransplantation between children and parents or *vice versa*.¹ It was of interest to determine whether in case of brother to brother transplantations within the inbred family the results would equal those of autotransplantation, and whether the individuality differentials between brothers in the inbred family had become identical.

We carried out two series of experiments in this direction; in the first one, series A, we used individuals belonging to the same family and in series B we exchanged tissues between brothers which were hybrids, the parents belonging to different inbred families.

SERIES A:

1. From 35-23-8 (16715) to 35-23-8 (16716) 5 months, 16 days. Thyroid and parathyroid behave like autotransplants. Grade 6.

2. From 32-19-9 (16900) to 32-19-9 (16899) 4 months, 9 days. Grade 6 (included among successive transplantations).

3. From 2N-26 (15673) to 2N-26 (15674) 61 days. Grade 6. Thyroid with auto-structure, acini close together. Solid retracted colloid, medium-sized epithelium. In one place there is a small number of lymphocytes. Cartilage well preserved, surrounded by areolar and fat tissue. No lymphocytes.

6. From hybrid 35 plus 32 to hybrid 39 plus 13. 35 days. No thyroid left. Cartilage surrounded by dense fibrous tissue; marked lymphocytic infiltration. Proliferative zone of cartilage near bone necrotic. Bone marrow replaced by fibrillar connective tissue and lymphocytes; capillaries with connective tissue penetrating into bone. Grade 1.

Average grade in this series is 2.

SERIES B. *Transplantation in control stock B.*

1. B-16854 to B 16870. 24 days. No thyroid (fibrous tissue only); cartilage mostly surrounded by fibrillar connective tissue, with much lymphocytic infiltration. Grade 1.

2. B-16870 to B-16854. 24 days. Grade 1.

3. B-16854 to B-16870. 24 days. Grade 1.

4. B-16870 to B-16854. 24 days. Grade 1.

5. B-16853 to B-16872. 25 days. Thyroid: around necrotic connective tissue some acini surrounded by fibrous tissue with no, or very little, colloid. Lymphocytes infiltrate acini. Cartilage surrounded by fibrillar connective tissue and areolar tissue with fibrous septa. Lymphocytic mantle around cartilage. Grade 2.

6. B-15681 to B-15536. 25 days. Thyroid very small. In fibrous tissue, infiltrated with lymphocytes, are small lumina of acini with remnants of colloid. Epithelium hardly recognizable. Fibrillar connective tissue with lymphocytes surround these acini, which are in process of destruction; many have already been destroyed. Hyaline fibrous tissue in center. Cartilage preserved, surrounded by fibrous tissue with variable amounts of lymphocytes. Remnants of areolar tissue. Lymphocytes penetrate a little into peripheral cartilage. Grade 2.25.

Average grade, 1.37.

SERIES C. *Transplantation from B stock to unrelated St. Louis stock.*

1. B to Bu. 40 days. Compressed remnants of thyroid; lymphocytic infiltration. Fibrous tissue around cartilage. Lymphocytes penetrate into periphery of cartilage. Grade 2.

2. B to Po. 20 days. Lymphocytes penetrate into periphery of perichondrium. Grade 1.

3. B to Po. 30 days. No thyroid. Lymphocytes penetrate in places into cartilage. Grade 1.25.

4. From Y-4 $\left\{ \begin{smallmatrix} 13-20-2 \\ 13-19-5 \end{smallmatrix} \right\}$ (16039) to Y-4 $\left\{ \begin{smallmatrix} 13-21-2 \\ 13-19-5 \end{smallmatrix} \right\}$ (16038) 40 days.

Transplant with striated muscle well preserved. Grade 6. (The corresponding transplantation to a relative a little further removed produced a syngenesio-reaction.)

5. Thyroid; cartilage and salivary gland from 2N-20 (15618) to 2N-20 (15619) 39 days, Grade 6.

6. From 2-18-4 (15693) to 2-18-4 (15692) 49 days. No connective tissue, new formation, nor lymphocytes. Grade 6.

7. From 13-19-9 (15649) to 13-19-9 (15648) 35 days. Thyroid with auto-structure, but in places in center and between acini around vessels are masses of lymphocytes surrounding and destroying acini. In a corresponding transplant in more distantly related animals of the same kind, there is much more lymphocytic infiltration than in brother to brother transplantation. Cartilage partly necrotic. Regenerating perichondrial cartilage infiltrates and replaces the necrotic cartilage. Connective tissue grows as papillae into necrotic cartilage, and the individual connective tissue cells grow also singly into it. Bone seems to have been produced in places where there was necrotic cartilage. In areolar tissue around cartilage some increase in connective tissue. Some megalokaryocytes, new bone marrow. Grade 5.

8. From 32-17-10 (15611) to 32-17-10 (15609) 40 days. Grade 6.

9. From 32-17-11 (15575) to 32-17-11 (15574) 10 days (died). Grade 6.

10. From 2-17-10 (15959) to 2-17-10 (15960) 50 days. Grade 6.

11. From 2-17-5 (15695) to 2-17-5 (15696) 30 days. Grade 6.

12. From 2-17-5 (15695) to 2-17-5 (15694) 30 days. Grade 6.

13. From 2N-20 (15617) to 2N-20 (15616) 12 days (died). Thyroid: very little lymphocytic reaction, but in places around the vessels there are small collections of lymphocytes. Cartilage similar to autotransplant. Grade 5.25.

14. From 2N-20 (15617) to 2N-20 (15619) 45 days. Grade 6.

Comment. In these 14 experiments the examinations were made after five months, sixteen days; four months, nine days; 61, 50, 49, 45, 40, 40, 39, 35, 30, 30, 12 and 10 days. In twelve cases the grade was 6, which means that the pieces showed the character of autotransplants; the individuality differentials between brothers in the same inbred family were identical as far as this test indicates. But this

in all cases, although on the whole they closely resemble one another. The figure for the average is here higher than in the case of the exchange of tissues in the same family between individuals which belonged to the same inbred family, but were not nearly related. In the latter case the average grade was 5.28.

IV. SYNGENESIOTRANSPLANTATIONS (BROTHER TO BROTHER) IN NON-INBRED FAMILIES

We have previously analyzed syngenesiotransplantation in non-inbred families of guinea-pigs. We found as the average grade of brother to brother transplantation 3.6. We shall here briefly state the result of brother to brother transplantation in the B strain of guinea-pigs for comparison with the results obtained in brother to brother transplantation within inbred families.

There were fifteen experiments; examination took place 25 to 40 days following transplantation. Grades: 6; 5.5; 5; 5; 5; 4.75; 3.75; 3.25; 3; 2; 2; 2; 1; 1; 1. Average grade 3.35.

A few examples may suffice. 1 B-202 to B-202, 35 days. No thyroid found. Fibrous tissue surrounds cartilage which is partly cellular. Marked lymphocytic mantle around cartilage, but in places loose connective tissue with fewer lymphocytes. The latter penetrate into cellular cartilage. Epithelioid reaction in fat tissue.

2. B-274 to B-274. 40 days. Thyroid very well preserved; good acini, close together with good solid restricted colloid. A little connective tissue in center; also a little areolar tissue with a small number of lymphocytes in one place in center. Cartilage well preserved surrounded by areolar tissue; no lymphocytes. Grade 6.

3. B-261 to B-261. 35 days. Thyroid. Remnants of acini, some containing colloid, surrounded by a mass of lymphocytes. Apparently remnants of parathyroid infiltrated by lymphocytes; especially in center of parathyroid much lymphocytic infiltration. Periphery of ring of acini surrounded by lymphocytes. Well preserved cartilage; where it is thicker, it is partly shrunken. Cellular cartilage is surrounded by areolar tissue which includes strands of connective tissue and lymphocytes, especially near the perichondrium and around vessels. However, the greater part of areolar tissue contains no lymphocytes. Grade 3.25.

Comment. The average grade for the syngenesiotransplants (brother to brother transplantations in non-inbred families) is con-

4. C-O-268 $\left\{ \begin{smallmatrix} 32-16-9 \\ 39-14-17 \end{smallmatrix} \right\}$ (16023) to C-O-268 $\left\{ \begin{smallmatrix} 32-16-9 \\ 39-14-17 \end{smallmatrix} \right\}$ (16025)
35 days. Thyroid and cartilage. Grade 6.

5. C-O-252 $\left\{ \begin{smallmatrix} 13-14-13 \\ 32-15-14 \end{smallmatrix} \right\}$ (16795) to C-O-252 $\left\{ \begin{smallmatrix} 13-14-13 \\ 32-15-14 \end{smallmatrix} \right\}$ (16794)
25 days. Thyroid approaching autotransplant; some slight diffuse lymphocytic infiltration in center; also in small peripheral area some lymphocytes. In places around cartilage a slight amount of newly formed connective tissue. Grade 5.5.

6. Reciprocal to (5): 16794 to 16795. Thyroid and cartilage, 25 days. Grade 6.

7. C-O-234 $\left\{ \begin{smallmatrix} 2-13-7 \\ 35-16-20 \end{smallmatrix} \right\}$ (17078) to C-O-234 $\left\{ \begin{smallmatrix} 2-13-7 \\ 35-16-20 \end{smallmatrix} \right\}$ (17079)
35 days. Thyroid like autotransplant. A small number of lymphocytes around vessels in center, probably within the range of that found in autotransplantation. Cartilage like autotransplant. Grade 6.

8. C-O-285 $\left\{ \begin{smallmatrix} 35-21-2 \\ 32-15-15 \end{smallmatrix} \right\}$ (16756) to C-O-285 $\left\{ \begin{smallmatrix} 35-21-2 \\ 35-15-15 \end{smallmatrix} \right\}$ (16757)
26 days. Thyroid like autotransplant; cartilage perhaps with slightly increased connective tissue and with some lymphocytes. Grade 5.75?

Comment. In these eight experiments the time of examination varied between 40 and 25 days. In five experiments the grade was 6. The hybrids in these cases were combinations of 39 plus 2; 39 plus 32; 13 plus 32; 2 plus 35. In three cases the transplants were not identical with autotransplants. The combinations and grades in these cases were as follows: 13 plus 32, grade 5; 13 plus 32, grade 5.5; 35 plus 32, grade 5.75. In the case of the last of these hybrids, experiment 8, it is doubtful whether grade 6 has not been reached. In experiments 5 and 6 (hybrid 13 plus 32) reciprocal transplants did not give exactly the same result, although the reactions in both cases were similar. It is again those hybrids, into whose composition family 13 entered, that do not yet reach the identity of individuality or 5.91 if we exclude experiments III A-13 and III B-1 differentials in brothers. The average grade in this series is 5.78, 5.89 if we omit experiment III B-1, or 5.91 if we exclude experiments III A-13 and III B-1. The average grade in series A and B combined is 5.84. These average grades also indicate that a perfect identity of the individuality differentials has not yet been reached.

acini and penetrate into thyroid in places; lymph vessels filled with lymphocytes. The greater part of the thyroid is intact, but in the connective tissue around acini there is much lymphocytic infiltration. Lymphocytes penetrate also into colloid. There are areas of fibrous tissue, of areolar tissue and of lymphocytic infiltration around cartilage. Striated muscle tissue preserved at one end.

7. C-O-240 $\left\{ \begin{array}{l} 32-16-9 \\ 35-16-11 \end{array} \right\}$ (15943) to 32-19-9 (16901) 37 days. Grade 4.

8. Same to 35-23-8 (16716) 37 days. Grade 2.

9. C-O-297 $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$ (18063) to 35-12 (18589) 25 days. Grade 2.50.

10. Same to 2N-25 (18184) 25 days. Grade 3.

11. C-O-282 $\left\{ \begin{array}{l} 2-15-3 \\ 32-17-7 \end{array} \right\}$ (15567) to 32-19-8 (15633) 25 days. Grade 6.

12. Same to 2-17-5 (15696) 25 days. Grade 3.

13. C-O-237 $\left\{ \begin{array}{l} 39-14-10 \\ 2-13-7 \end{array} \right\}$ (15893) to 39-16-21 (15727) 25 days. Grade 2.5.

14. Same to 2-18-4 (15692) 25 days. Grade 2.85.

15. C-O-297 $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$ (18066) to 2N-31 (19998) 20 days. Grade 2.75.

Much thyroid tissue destroyed, relatively little thyroid left. The remaining ring of acini with much lymphocytic infiltration and much fibrous tissue formation. Around cartilage still some muscle tissue preserved.

16. Same to 35-22-17 (17966) died after 15 days. Grade 3.25. (Some muscle tissue preserved.)

17. C-O-298 $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$ (18190) to 13-21-8 (18185) 21 days.

Grade 1.25. Thyroid destroyed; merely dense fibrous tissue and some lymphocytes surrounded by fat tissue and epithelioid and giant cells. Bone marrow replaced by loose connective tissue. Cartilage mostly necrotic. Some fat tissue mingled with epithelioid and giant cells. Over wide areas cartilage surrounded by connective tissue with some lymphocytic infiltration. Near bone, a zone of cartilage is living.

18. Same to 2N-32 (20338) 21 days. Grade 2.

19. C-O-269 $\left\{ \begin{array}{l} 32-16-9 \\ 39-14-17 \end{array} \right\}$ (16025) to 32-19-8 (15633) 23 days.

Grade 5.5. Large thyroid with solid retracted colloid; medium-sized

siderably lower than the average grade of ordinary homoio-transplantations in the inbred families and still lower than the grade for the brother to brother transplantations in the latter.

V. TRANSPLANTATION FROM HYBRID TO PURE COMPONENT FAMILY

In this series a male belonging to one of the inbred families and a female belonging to a different family were mated, and the hybrid thus obtained was used for transplantation to another individual who was not related to the hybrid but belonged either to the family of his father or mother. Thus twenty-four experiments were carried out. The length of time during which the pieces were left in the host varied between 37 and 20 days. In one additional case the host died 15 days after transplantation.

The relationships between host and donor will be brought out more distinctly in the following list of experiments:

1. C-O-282 $\left\{ \begin{array}{l} 2-15-3 \\ 32-17-7 \end{array} \right\}$ (15566) to (32-17-11 (15575) 24 days.

Grade 4.25. Thyroid: structure of autotransplant, but dense lymphocytic masses in center and in places in peripheral fibrous capsule around vessels. Lymphocytes penetrate from center towards periphery between acini. Many acini have been destroyed. Some connective tissue increase in center and around acini. Cartilage: well preserved surrounded by areolar tissue with slight increase in connective tissue and lymphocytes.

2. Same to 2N-20 (15618) 24 days. Grade 4.

3. C-O-298 $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$ (18188a) to 13-20-13 (15784) 35 days. Grade 2.

4. Same to 2-16-17 (18059) 35 days. Grade 1.

Thyroid: only fibrous tissue found. Cartilage: with a great deal of necrosis; some shrunken cells. Much connective tissue and moderate mantle of lymphocytes around cartilage, the necrotic parts of which are entered by some connective tissue and lymphocytes.

5. C-O-304 $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$ (18305) to 32-18-15 (16753) 37 days. Grade 3.75.

6. Same to 2N-24 (18043) 37 days. Grade 3.25.

Thyroid: very good acini, close together in places and with solid retracted colloid. Much lymphocytic infiltration around and between acini. Also in center of thyroid lymphocytes surround some

$\frac{2}{35}$ was used in five experiments. (9) $\frac{2}{35}$ to 35, 25 days. Grade 2.50. (10) $\frac{2}{35}$ to 2N, Grade 2. (14) $\frac{2}{35}$ to 2N, 20 days. Grade 2.75. (15) $\frac{2}{35}$ to 35 (died after 15 days). Grade 3.25. (24) $\frac{2}{35}$ to 2, 31 days. Grade 4. In these experiments the results were almost but not quite as unfavorable as in transplantation for hybrid $\frac{13}{2}$.

$\frac{39}{2}$ was used in two experiments. (12) $\frac{39}{2}$ to 39, 25 days. Grade 2.5? (13) $\frac{39}{2}$ to 2, 25 days. Grade 2.75-3. In this combination the results were likewise unfavorable.

Hybrid $\frac{32}{39}$ was used in four experiments. (19) $\frac{32}{39}$ to 32, 23 days. Grade 5.5. (20) $\frac{32}{39}$ to 39, 23 days. Grade 1.25. (22) $\frac{39}{32}$ to 39, 25 days. Grade 3.25. $\frac{39}{32}$ to 32, 25 days. Grade 5. In this combination the results were relatively favorable.

In three experiments, the result approaches that in autotransplantation; in one experiment the result corresponded to a favorable syngenesiotransplantation. In nine cases the result was a very pronounced syngenesio-reaction. In three cases the result was on the border-line between a syngenesio- and homoio-reaction and in eight experiments a homoio-reaction was obtained. The average grade was 3.25. The average grade was therefore only very slightly less favorable than that obtained in brother to brother transplantation in non-inbred families.

We see thus that the grades in the different experiments vary as much as between 6 and 1. The grades were as follows: 6; 5.5; 5.5; 5; 4.25; 4; 4; 4; 3.75; 3.25; 3.25; 3.25; 3; 3; 2.85; 2.75; 2.50; 2.50; 2; 2; 2; 1.5; 1.25; 1.

In eleven experiments we transplanted the organs of one hybrid to both the component parent strains; in none was the transplantation made to the direct parents of the hybrids. In seven of these experiments the results were similar after transplantation into both parent strains, in three experiments they were very different, and in

epithelium. Many large acini. A small amount of connective tissue in center. No lymphocytes except in one connective tissue septum, where there is a slight collection. Well preserved parathyroid. Cartilage well preserved, surrounded by areolar tissue. There is a slight increase in connective tissue with very little lymphocytic infiltration in areolar and fat tissue.

20. Same to 39-16-21 (15633) 23 days. Grade 1.25.

21. C-O-304 $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$ (18306) to 2N-25 (18182) 29 days. Grade 5.5.

22. C-O-284 $\left\{ \begin{array}{l} 39-13-13 \\ 32-17-5 \end{array} \right\}$ () to 39-16-21 (15727) 25 days. Grade 3.25.

23. Same to 32-17-11 (15574) 25 days. Grade 5.

24. C-O-297 $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$ (17093) to 2-17-5 (15696) 31 days. Grade 4.

The hybrid combinations used were as follows: $\frac{2}{32}$ to 2 in four different experiments; $\frac{2}{32}$ to 32 in three different experiments.

(1) $\frac{2}{32}$ to 32, 24 days. Grade 4.25. (2) $\frac{2}{32}$ to 2N, 24 days.

Grade 4. (5) $\frac{32}{2}$ to 32, 37 days. Grade 3.75. (6) $\frac{32}{2}$ to 2N, 37 days.

Grade 3.25. (11) $\frac{2}{32}$ to 32, 25 days. Grade 6. (18) $\frac{2}{32}$ to 2. 25 days.

Grade 3. (21) $\frac{32}{2}$ to 2N, 29 days. Grade about 5.5.

$\frac{13}{2}$ was used in four experiments. (3) $\frac{13}{2}$ to 13, 35 days. Grade 2.

(4) $\frac{13}{2}$ to 2, 35 days. Grade 1. (16) $\frac{13}{2}$ to 13, 21 days. Grade 1.5.

(17) $\frac{13}{2}$ to 2N, 21 days. Grade 2. In transplantations in which family 13 enters as a component of a hybrid, the reactions against the transplants are more severe than in transplantations in which family 32 takes the place of family 13.

$\frac{32}{35}$ was used in two experiments. (7) $\frac{32}{35}$ to 32, 37 days. Grade 4.

(8) $\frac{32}{35}$ to 35, 37 days. Grade 2.

families are possibly less sensitive to differences in genes than other families. The experiments recorded so far in these series suggest these conclusions.

VI. TRANSPLANTATION FROM PURE COMPONENT FAMILY TO HYBRID

In this series the reverse transplantations were carried out. Tissues were transferred to a hybrid from an individual belonging to one of the two inbred families, members of which had been used for hybridization. Host and donor were not directly related to each other, except in so far as all members of an inbred family are in certain respects so related.

Fourteen experiments were carried out in this series; the period during which the transplants remained in the host varied in the different cases between 25 and 35 days.

The following experiments were made:

1. 2N-25 (18184) to C-O-297 $\left\{ \begin{array}{l} 2-17-8 \\ 35-23-10 \end{array} \right\}$ (18063) 25 days.

Grade 6. Thyroid: with auto-structure; acinus cells of low to medium height, solid retracted colloid. Some loose connective tissue and large vessels in center. No lymphocytes, no increase in connective tissue. Cartilage well preserved; normal fine fibrillar connective tissue and fat tissue surround the cartilage. Again no lymphocytes; no increase in connective tissue.

2. 351-2 (18589) to same hybrid, 27 days. Grade 5.25. Thyroid, as in preceding experiment, but some masses of lymphocytes penetrate from outside to center. Cartilage also well preserved and surrounded by fat tissue, but in places some fibrillar connective tissue with a few lymphocytes around it. In this case a very slight reaction took place.

3. 2-16-17 (18059) to C-O-298 $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$ (18188a) 35 days.
Grade 6.

4. 13-21-13 (15784) to same hybrid. Grade 6.

5. 32-18-15 (16753) to C-O-304 $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$ (18305) 35 days.

Grade 6. Only a small collection of lymphocytes around foreign body; otherwise like autotransplant.

6. 2N-24 (18043) to same hybrid. 35 days. Grade 6. As in the majority of other transplants, the perichondrium produces in places

one case the difference, although noticeable, was not quite so pronounced. Different results were obtained in the following combinations: $\frac{2}{32}$ to 32, grade 6. $\frac{2}{32}$ to 2 grade 3. $\frac{32}{35}$ to 32, grade 4. $\frac{32}{35}$ to 35, grade 2. $\frac{32}{39}$ to 32, grade 5.5. $\frac{32}{39}$ to 39, grade 1.25. $\frac{39}{32}$ to 39, grade 3.25. $\frac{39}{32}$ to 32, grade 5.

In general it seems that hybridizations, into which family 32 enters as one of the strains, are relatively favorable; the reactions on the whole are slight, at least in a number of these cases, and especially when the host belongs to family 32. The presence of a single dose of 2, 35 or 39 family-differential does not necessarily lead to severe reactions if a guinea-pig belonging to family 32 is the host, but in other cases it may do so. If a member of family 2 is host, the reactions against hybrid tissue $\frac{32}{2}$ are usually more marked than the reactions on the part of a 32 host, but here also they may be very slight. Similarly host 32 reacts much less actively against $\frac{32}{35}$ hybrid tissue than a 35 host. In host 35 the reactions are pronounced against hybrid tissue of 35 with 2 or 32. Host 32 showed relatively slight reactions against $\frac{32}{39}$ hybrid tissue, while host 39 showed decidedly more marked reactions against the same tissue. Host 2 showed quite noticeable reactions against $\frac{2}{35}$ and $\frac{2}{39}$ hybrid tissues. Among the most marked reactions were those obtained against hybrid tissue in which 13 entered as a component. The reactions occurred in host 2 as well as in host 13. Throughout our experiments we found the most marked reaction within family 13, which represents probably the least homozygous of the various families. Not only is the reaction against family 13 most marked on the part of other families, but family 13 as host reacts likewise most actively against the individuality differential of the other families. We may assume that the different families show a varying degree of relationship to each other, and that while the strange genes in general cause a reaction on the part of the host, perhaps certain genes call forth a more severe reaction than others, and furthermore that certain

range of lymphocytic collections found in autotransplants. In the cartilage, some regenerated transplanted muscle tissue with chains of nuclei. There is a very slight increase of connective tissue with some lymphocytes found in the areolar and fat tissue around cartilage.

13. 2N-20 (15619) to C-O-297 $\left\{ \begin{smallmatrix} 2-17-8 \\ 35-23-10 \end{smallmatrix} \right\}$ (18066) 31 days.

Grade 5.50. Thyroid like autotransplant, except for a few small collections of lymphocytes. In cartilage transplant there is some transplanted striated muscle with only a few nuclear chains. In places there are small collections of lymphocytes around vessels in fat tissue of cartilage transplant probably exceeding the number found in autotransplants.

14. 35-22-17 (17695) to same hybrid. 31 days. Grade 5.25. Thyroid resembles autotransplant except that there is some thickening of connective tissue septa and a slight lymphocytic infiltration. In cartilage transplant, which is well preserved, there are some distinct collections of lymphocytes in areolar and fat tissue and there is here also a very slight increase in connective tissue. Areas of necrotic cartilage are partly replaced by perichondrial cartilage which may regenerate in the form of a plate. Near the junction of xiphoid cartilage with bone, connective tissue grows into areas of necrotic cartilage and produces a bone-like substance. We find also fibrillar bone marrow with megalokaryocytes, capillaries and lymphocytes. Osteoclasts also are seen at the edge of bone marrow. Between the areas of bone, perichondrium forms cartilage. A somewhat similar condition was probably observed in experiment (8) of this series. In this transplant very well formed muscle tissue produced by transplanted muscle cells was seen.

Comment. The average grade in these fourteen experiments is 5.77, approaching therefore the condition found in autotransplantation. In eight cases the grade corresponds to the result obtained in autotransplantation. In the remaining experiments the grades are 5.75; 5.50; 5.50; 5.50; 5.25; 5.25. Grade 6 was obtained in the following transplantations: 2 to $\frac{2}{35}$; 32 to $\frac{32}{2}$ in two experiments; 2 to $\frac{32}{2}$ in two experiments; 32 to $\frac{32}{35}$; 13 to $\frac{13}{2}$ and 2 to $\frac{13}{2}$. The other grades were obtained in the following combinations: 35 to $\frac{2}{35}$ in two

new cartilage, which either penetrates adjoining necrotic cartilage and replaces it or which is deposited in the form of a plate.

7. 32-19-9 (16900) to C-O-240 $\left\{ \begin{array}{l} 32-16-9 \\ 35-16-11 \end{array} \right\}$ (15943) 35 days.

Grade 6. Transplants resemble autotransplants. Into peripheral necrotic cartilage some connective tissue is growing and in places it penetrates superficially even adjoining living cartilage. A very few small strands of lymphocytes in fat tissue; conditions are probably still within the range of those observed in autotransplants.

8. 35-23-8 (16716) to same hybrid. 35 days. Grade 5.75. Thyroid and parathyroid like autotransplants, except that we find in the parathyroid in one place a slight, but distinct collection of lymphocytes which exceeds in size collections found in cases of autotransplantation. In areolar and fat tissue around cartilage there are few polymorphonuclear leucocytes and lymphocytes. There may also possibly be here and there a very slight increase in connective tissue.

9. 32-18-9 (18179) to C-O-304 $\left\{ \begin{array}{l} 32-19-10 \\ 2-15-16 \end{array} \right\}$ (18306) 25 days. Grade 6.

10. 2N-25 to same hybrid 25 days. Grade 6. Thyroid transplant consists of ring of well preserved acini with a small amount of connective tissue and areolar tissue in center. Colloid in acini is solid and somewhat retracted. Cartilage is surrounded by areolar and fat tissue.

11. 13-18-10 (16788) to C-O-298 $\left\{ \begin{array}{l} 13-21-4 \\ 2-16-17 \end{array} \right\}$ (18190) 32 days.

Grade 5.50. Thyroid, parathyroid and cartilage like autotransplants, except for a collection of lymphocytes in periphery of parathyroid which also penetrates into this gland. Cartilage is well preserved, but parts are necrotic; the latter are surrounded by regenerated perichondrial cartilage which as usual consists of small cartilage cells with nuclei that are more prominent than in older cells, its blue stain contrasting within the light stain of the cytoplasm. In other places the host connective tissue grows into necrotic cartilage and replaces it. Some lymphatics in fat tissue are filled with lymphocytes.

12. 2N-25 (18182) to same hybrid. 32 days. Grade 5.50. Thyroid like autotransplant except that in one place in the center there is a small mass of lymphocytes which probably exceeds in size the

series is slightly lower than the average we obtained in the last series in which we transplanted pieces from a component strain to a hybrid; here the grade was 5.77. Of course the slight difference between these figures may have no significance. The figure for the last series is about the same as that for the series in which tissues were exchanged between two brothers, which were both hybrids of the same inbred families; here the average grade is 5.78. In this case the presence of two component families in donor as well as in host is an unfavorable condition which is compensated by the fact that both host and donor are brothers. In general we have some indication that transplantations from one hybrid to another not directly related hybrid, both derived from the same families, call forth a stronger reaction and give a lower average grade (tentatively 4, a figure based however on a very small number of experiments) than transplantations within the same inbred family, if host and donor are not directly related. Transplantations from brother to brother in the inbred families are closer to autotransplantations than any of the preceding kinds of transplantations, the average grade being 5.9; this of course is in accordance with expectations. On the other hand, the transplantations from a hybrid to the component strains call forth strong reactions; the average grade is accordingly low, namely 3.25, which is very similar to the average grade in brother to brother transplantations in the B group, in which the average is 3.35.

CONCLUSIONS

From these results we may conclude that it is not the similarity or difference between individuality differentials of donor and host which determines the reaction against the transplant, but the reaction depends on the presence in the host of genetic factors of the donor. The lack in the donor of genetic factors present in the host is apparently of little or no consequence. In the case of tumor transplantations in different varieties of mice which have been inbred, although as it seems not exclusively through consecutive brother to brother matings, Little and Tyzzer³ found that transplantations from hybrid to parent strain gave negative results as far as the number of takes was concerned, while the reverse transplantation gave 100 per cent takes. They concluded therefore that only one dose of genes is required for successful transplantation. We found in our experiments, in which we used a much finer means of measuring genetic

experiments 2 to $\frac{2}{35}$; 13 to $\frac{13}{2}$ and 2 to $\frac{13}{2}$. Slight reactions were therefore observed in cases in which family 13 or family 35 were hosts or donors. The strongest reactions were obtained in cases in which 35 was both host and donor, and especially in experiments in which family 35, serving as host, was combined with family 2. When families 32 and 35 were combined as host and donor a reaction, although somewhat mitigated, was observed. As in the preceding series we found that hybridizations, in which families 13 and 35 were involved, gave rise to relatively stronger reactions if tissues were exchanged between hybrids and component families than if the transplantations concern families 32 and 2. In this series we have not used family 39. On the other hand, we find that exchange of tissues in cases in which families 13 and 35 enter may not necessarily give rise to reactions in all cases; the individuality differentials between donor and host may be so constituted that no reaction occurs, at least within the range of time used in the particular experiment.

These fourteen experiments may be arranged in seven groups in which an exchange of tissue took place between hybrid members of the two families in each case. In four of these groups the results were about the same without regard to the character of the donor family; in three there was some difference, in each instance the stronger reaction was obtained when family 35 furnished the donor.

If we compare the average grades in the different series, omitting those in which the number of experiments at present is still very small, we find in the transplantations within the same family an average of 5.6 or slightly higher. The multiple transplantations extending over a long period of time give a somewhat lower average, namely 5.4, as do successive transplantations, although the number of our experiments is very small in this latter subseries. Some complicating factors may enter in the case of multiple transplantations extending over a long period of time, and of successive transplantations.

In experiments in which we transplanted from one hybrid to another hybrid of the same composition, not directly related, stronger reactions seemed to be elicited, and the averages were correspondingly lower, namely grade 4; but here again the number of our experiments is as yet too small.

The average grade of 5.6 in the uncomplicated subdivision of this

Apparently the absence of genes in the transplant is without significance. It is the presence of strange genes in the transplanted tissue which causes the inferiority in the latter in respect to the survival and regenerative power.

Both of these consequences of transplantations of tissues, in which the individuality differentials are more or less incompatible with those of the host, agree with those established previously by one of us in his series of transplantations. The lymphocytic and connective tissue reactions, the behavior of blood vessels, the degeneration of sensitive tissues like bone marrow, liver cells and spleen tissue in accordance with incompatibilities of individuality differentials are the same in these experiments as those previously described.

As to other general conclusions, we may refer to the discussions given at the end of the various sections in this paper. We may again state that our observations point to the conclusion that in some families a homozygous condition has been reached more completely than in others, but that it has not yet been actually attained in any of these inbred families. It is probable that some families are more nearly related to each other than others and furthermore it is possible that certain strange factors present in some families and individuals cause a greater incompatibility reaction than others and, in addition, we must consider the possibility that the sensitivity and degree of reaction of certain families or individuals against individuality differentials surpass those of others.

There is some indication that in the transplantations within the same family, the individuality differentials of host and transplant are more similar to each other the larger the number of brother and sister matings which both individuals had in common before the separation of brother and sister matings in different sidelines and the smaller the number of these separate and distinct brother and sister matings for each of the two individuals in the preceding generations. Thus in family 2, three transplantations with an average grade of 4.6 were separated for twenty-four, twenty-four and twenty generations of brother and sister matings respectively; while preceding their separate matings they had six, six and nine common ancestral matings. In two other experiments in this family in which host and donor were separated for twelve and three generations only, and twelve and sixteen brother and sister matings in common, the grade was 6.

composition than tumor transplantations, that in different transplantations from hybrid to component strain as well as from component strain to hybrid, individual differences exist which are very pronounced in the case of transplantations from hybrid to component strain. It is probably not so much the presence of a single dose of the genes which is required, as far as the absence of reaction against the transplant is concerned, as the lack of a strange gene in the donor; while the presence of a strange gene in the host is without significance. Thus the transplantation from one hybrid to another hybrid is more injurious than transplantation from component family to hybrid.

More recently, Little and Johnson⁴ found in an inbred strain of Japanese waltzing mice that transplantation of pieces of spleen from waltzing mice to hybrids of these animals with white mice, behaved like autotransplants; while spleen transplanted from such hybrids to waltzing mice behaved like very pronounced homoiotransplants, and were destroyed within a short time. In these experiments the transplantations in which the hybrids served as hosts gave much better results than the reciprocal transplantations; but again the lack of finer means of measuring genetic differences led in all probability to incorrect estimates of the genetic identity or lack of identity in the individuality differentials of host and donor. It is very probable that the results in these transplantations did not correspond exactly to those found in autotransplantation, or to those of complete homoiotransplantation, but that both were intermediate between these two extremes. However the fact brought out by Little and Johnson and also, it seems, their interpretation of these facts, appear to be in essential harmony with the conceptions which one of us had previously developed concerning the individuality differential.

The results which we obtained cannot be considered inevitable from *a priori* reasoning. Thus one might have expected that the presence in the individuality differential of the host of a set of genes which are not present in the donor and transplanted tissue should lead to essential differences between the metabolism of host and transplant and consequently to aggressive reactions on the part of the host; this evidently does not take place. It seems to be merely the presence of strange genes in the transplanted tissue which acts as a stimulus on the host and incites the cells of the latter to react.

ilies used for transplantation, and represent the relationship between the differentials.

Autotransplantation, grade 6.

Homoiotransplantation, grade 1.6.

Transplantation within inbred families, grade 5.5.

Multiple transplantation within inbred families, grade 5.15.

Successive transplantations within inbred families, grade 4.8 (?) (few experiments).

Transplantation from brother to brother in non-inbred families: grade 3.35.

Both com- bined: 5.84 (5.91)	{	Transplantation from brother to brother in inbred families: grade 5.87 (5.92).
	{	Transplantation from brother to brother, hybrids of inbred families: grade 5.78 (5.89).
		Transplantation from component families to hybrid between inbred families: grade 5.7.
		Transplantation from hybrid between inbred families to component families: grade 3.24.

2. Within the different inbred families the individuality differentials have reached a very great similarity. The resemblance of the individuality differentials among members of an inbred family which are not closely related is much greater than that among brothers in non-inbred families.

3. There is an identity of the individuality differentials. A complete loss of individuality has not yet been reached within the inbred families. In general the individuality differentials within an inbred family seem to approach more nearly identity the greater the number of generations of brother and sister matings they had in common and the smaller the number of generations since they split off into sidelines of generations of brother and sister matings. On the whole, brothers and sisters seem to have reached identity of individuality differentials after nineteen to twenty generations of continuous brother and sister matings; at least no reaction was elicited in the host on the part of the transplant within the range of time used in our experiments. However, in a few cases there was observed a lack of complete identity of individuality differentials even under these conditions. It is probable that within certain families the homo-

A similar result was obtained in matings in family 13. Here the average grade was 4.9 in a series of transplantations in which the number of generations of brother and sister matings since the splitting off of the sidelines of brother and sister matings varied between twenty-three and twenty-seven, while the average number of preceding brother and sister matings which both individuals had in common was slightly less than nine. In three other transplantations in which the number of generations since separation was about twelve, and the number of common generations varied between thirteen and seventeen, the average grade was 5.8. Which of these two factors, the number of generations which donor and host had in common, or the number of separate generations of brother and sister matings in these two individuals is the important one, is impossible to decide on the basis of our data. If the latter factor should play an important part it would be necessary to attribute this effect to possible mutations which occurred during the period of separate breedings: the mutations under these conditions would then have been different in the ancestry of host and donor.

In the large majority of cases in which after nineteen or twenty consecutive brother and sister matings, tissues were transplanted from brother to brother or sister the reactions taking place showed absence of incompatibility, and therefore at least apparent identity between individuality differentials of host and donor. It is therefore probable that under these conditions the difference between the individuality differentials of host and donor disappeared. These two individuals should therefore behave like identical twins. That this condition has actually been reached cannot yet be definitely asserted, inasmuch as it is possible that a slight reaction might still have appeared at a later date. But, on the other hand, we can be certain that in two cases in which such consecutive brother and sister matings had taken place the individuality differentials of brothers had not yet reached identity.

SUMMARY

1. The principal results obtained in these investigations are represented in the following list of average grades expressing the reactions of the hosts against transplants. These reactions depend upon the constitution of the individuality differentials of the respective fam-

geneity of individuality differentials of the various members of the family is greater than within other families. It is also probable that the average individuality differential of a certain inbred family may differ more from the average individuality differentials of various other inbred families than these differ from each other.

On this basis it would be possible to explain the stronger reactions which tissues of family 13 call forth when they are transplanted into other families, as well as the strong reaction of family 13 serving as host. In addition it is possible that certain strange genes call forth a more intense reaction than other strange genes, or that a host possessing certain genes is able to react more intensely to genes not represented in its own individuality differential.

4. In general the number of strange genes (and perhaps also the intensity of the strangeness of the composing genes) in the individuality differential of the transplanted tissue determines the severity of the reaction of the host against the transplant. The presence of strange genes in the individuality differential of the host; or expressed differently, the absence of certain genes in the transplant or the presence of double genes in the transplant, does not call forth a reaction in the host. On this basis, we can understand the difference between the results of the transplantation from component strain to hybrid, and of the reciprocal transplantation, as compared to the severity of reaction observed after the exchange of tissues within the same inbred family.

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globular bodies stain deeply with phosphotungstic acid, and constitute the familiar circular reticulum of the kidney so often seen in kidney sections. The true origin of this circular reticulum has not, I believe, been appreciated before now. The globular bodies are at times nearly devoid of cytoplasmic material. More frequently, however, they contain, particularly about their periphery, masses of deeply staining material derived from the parent cell.

Presumably under the influence of the urine, which in chronic nephritis is liable to be more acid than normal, these globular bodies partially disintegrate and the coarse masses of deeply staining material become compressed about clear circular areas once marked by the limiting membrane of the reticulum (Fig. 7). The cytoplasmic granules become finer, more densely massed together, and stain less intensely with phosphotungstic acid and more intensely with eosin. Evidences of the original arrangement can, however, still be seen (Fig. 8).

This comminuting process continues (Figs. 8 and 9) and the whole aggregate now resembles the form of the true cast though the surface is still granular, and the vacant circular areas can still be seen (Fig. 9). Finally the mass becomes truly homogeneous and stains very deeply with eosin. This is the immediate precursor of the true cast as seen in sections. It is probable that blood and epithelial cells may be caught in this coagulum as it passes down the tubules, and thus various types of casts are formed.

The different stages can be followed in succession from the earliest buds which are formed mainly in the upper portions of the convoluted tubules, through the various stages of degeneration of the circular reticulum, to the gradual comminution and coalescence of the granular *débris* which occurs in the loops of Henle and the collecting tubules, to the formation of a homogeneous cast.

This same process has been traced in human kidneys in cases of chronic nephritis. The swelling of the cells, which characterizes the earliest stage; and the later stages beginning with the circular reticulum to the formation of casts can be easily found, but the actual budding of the cells is infrequently seen. Only very careful examination under an oil immersion lens will reveal their presence. Typical buds have, however, been found in human tissue fixed very shortly after death.

It is not to be thought that this explanation is necessarily correct

THE HISTOGENESIS OF URINARY CASTS *

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The histogenesis of renal casts has for many years interested clinicians and clinical pathologists and much work has been done in investigating the problem. Some authors are largely speculative.^{1, 2} One³ explains the origin of casts on the basis of abnormal surface tension phenomena while others content themselves with broad generalities. Some years ago R. M. Smith⁴ in an admirable paper reviewed the literature to date and attributed the origin of urinary casts to the gradual fusion of desquamated epithelium in the lumen of the tubules. He presented photomicrographs to show the gradual coalescence of this necrotic mass as it passes down to the collecting portion of the kidney. No doubt this is true in certain cases, especially in acute nephritis, but the explanation would hardly seem adequate in chronic nephritis, where even in the presence of many casts there may be little or no desquamation of renal epithelium.

While studying the rats' kidneys rendered slightly nephropathic by long continued feeding of very high protein diets, certain cells were observed to be "budding" or extruding some portion of their substance into the lumen of the tubule. A careful examination of the sections revealed what appears to be the origin of at least some types of casts, and at the same time, of the circular reticulum of the kidney.

The first change to be seen (Fig. 1) is a swelling or vacuolization of the inner portion of the cells lining the convoluted tubules. The cell becomes edematous at its inner end. As the edema increases the cell swells still further and there is formed a very striking globular or pear-shaped bud (Figs. 2, 3 and 4) connected with the main cell body by a more or less attenuated stalk. Extending into this bud, especially along its periphery, cytoplasmic material may be seen. Occasionally a disrupted nucleus is present in the bud. Under the influence of whatever forces are at play, this bud is constricted off at its base and comes to lie free in the lumen of the tubule in the form of a globe several times the diameter of the cell nucleus (Fig. 6). These

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DESCRIPTION OF PLATES

PLATE 80

- FIG. 1. Cell of convoluted tubule showing the initial swelling and edema at the tip. $\times 3000$.
FIG. 2. Very early bud from renal cell. Note masses of cytoplasm carried into the bud. $\times 3000$.
FIG. 3. Later stage of bud-constriction at base. $\times 3000$.
FIG. 4. Constricting process nearly complete. $\times 3000$.

PLATE 81

- FIG. 5. Budding cell in gland of pregnant uterus. Note the nucleus passing into the bud. $\times 2000$.
FIG. 6. Circular reticulum of kidney. $\times 1000$.
FIG. 7. Dissolution of the reticulum with the coalescence of its granules. $\times 1000$.

PLATE 82

- FIG. 8. Further comminution of the granular debris from the reticulum. $\times 1000$.
FIG. 9. Granular mass lying in the lumen of tubule. Nearly homogeneous. Stains intensely with eosin. $\times 1000$.
FIG. 10. Cast lying free. Homogeneous mass with circular areas still visible. $\times 500$.

for all urinary casts. There may well be other modes of formation, especially in acute nephritis, but it would seem to offer an adequate and logical explanation at least in certain cases.

By virtue of what pathologic change in the cell or its environment the primary swelling and budding take place is a matter for conjecture. But the process is analogous to similar phenomena which have been observed by Chambers and others when single cells outside the body have been injured, and the process may well be looked upon as merely an expression of injury on the part of the renal cells. That such is indeed the case is further evidenced by the fact that similar if not identical buddings have been found in the cells lining the uterine glands during pregnancy (Fig. 5), and a pathologic picture indistinguishable from the circular reticulum has been found in the adrenal gland in certain pathologic conditions. It may well be supposed that under the influence of changes in cell environment such phenomena may occur in widely different organs, but that only in the kidney is the subsequent history of the reticulum that of development into a cast.

SUMMARY

Evidence is presented that at least certain casts in chronic nephritis are formed by the coalescence of granules found in the circular reticulum of the kidney, and that this reticulum is in turn formed by an abnormal budding of the renal cells. This process is not peculiar to the kidney, at least so far as the initial stages are concerned, for it has been seen in other organs under varied conditions. The reaction is to be interpreted as due to an abnormal environment about the cells involved.

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2



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Jackson

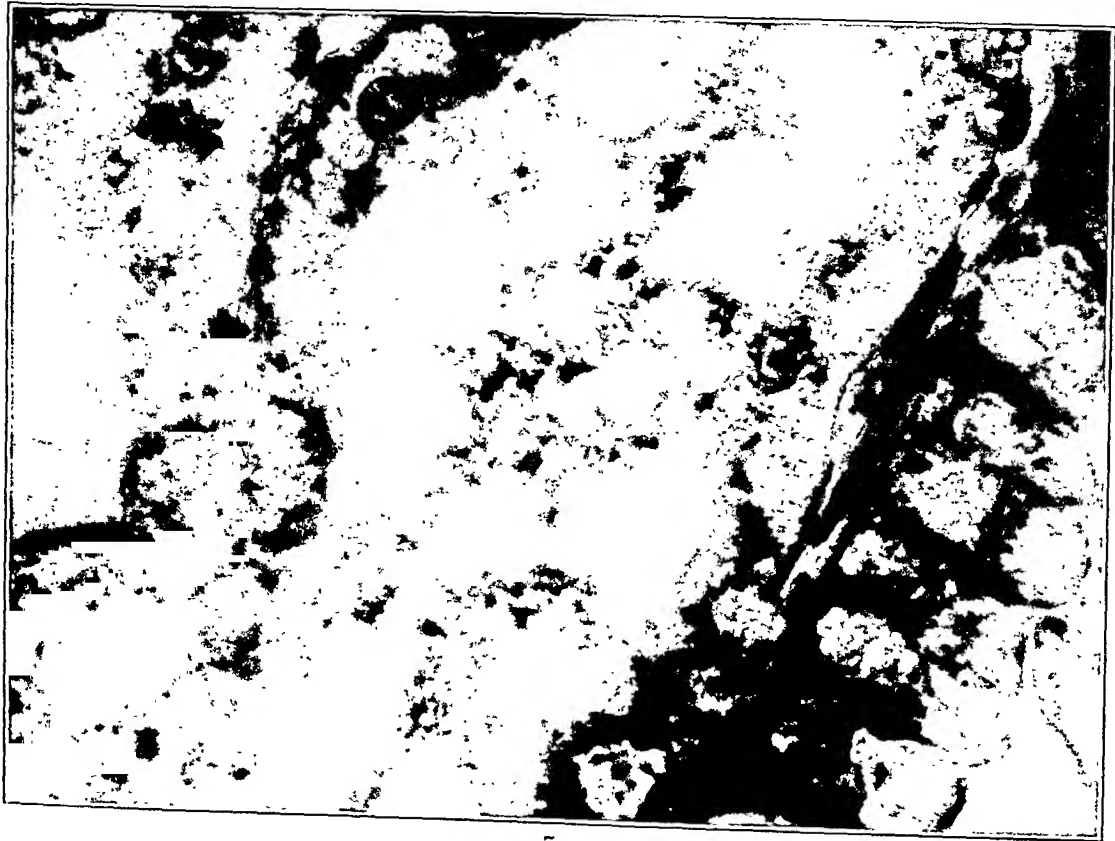
The Histogenesis of Urinary Casts



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Jackson

The Histogenesis of Urinary Casts

His present complaint apparently started about one year before entry, with a dull, aching, non-radiating pain in the right thigh which was worse at night. About one month later similar pains made their appearance across his chest, arms and shoulders, and became so severe that they prevented sleep. Six months later while walking down the road, and without any apparent cause, a spontaneous fracture of the right femur occurred. He was placed in a local hospital and a cast applied to the limb. However, during his five months' stay there, before entry to the University hospital, no union occurred in the fracture, the pains became progressively more intense and the patient noted that his right chest, especially the lower ribs, was gradually sinking in, causing considerable dyspnea.

Physical Examination: An emaciated man appearing older than the age stated, with a generalized brownish pigmentation of the skin. There were several non-tender nodules varying in size from a pea to a hazelnut scattered over the frontal bone, and a scar about the size of a half-dollar over the left malar region. Eyes, nose, and ears were negative except for slight impairment of hearing on the right side. The teeth showed some dental caries and pyorrhea, and there were a few pin-point hemorrhagic areas on the hard palate. There were no palpable glands in the neck. The chest presented a marked deformity on the right side with a funnel-shaped depression in the center (Fig. 1); dullness over the apex of the right lung, with increased vocal and tactile fremitus over the entire lung, and bronchovesicular breath sounds with moist râles at the base; dullness over the apex of the left lung, increased breath sounds and vocal and tactile fremitus over the whole lung and a friction rub over the base in the axillary line. The heart was negative, pulse rate 116, arteries normal, the blood pressure systolic 115 and diastolic 70. The abdomen showed a firm, smooth, slightly tender tumor mass with rather indistinct edges, occupying the left upper quadrant, extending down to the umbilicus and to the midline and moving with respiration. The liver dullness extended 4 cm. below the tip of the ensiform cartilage but the liver could not be felt below the lateral costal margin. Examination of the genitalia, rectum and prostate was negative. The extremities were emaciated, and the right thigh and leg which were splinted showed some slight edema. The reflexes were normal.

The laboratory findings, with the exception of the blood examination which is detailed in the chart below, were essentially negative. The blood Wassermann was negative and at no time was Bence-Jones albumose found in the urine.

Roentgenologic Examination: Numerous fractures of the ribs and transverse processes with many irregular areas of rarefaction in them and in the vertebral bodies. About two inches below the lesser trochanter of the right femur a T-shaped fracture was seen, with rounded ends and some callus formation. On the inner side of the right tibia, about the middle of the shaft, was another small irregular area of rarefaction (Figs. 2, 3 and 4).

On November 4, about 5 cm. of the right seventh rib was removed for diagnostic purposes. This was examined by Dr. E. I. Bartlett who reported that the weight of evidence was in favor of carcinoma, but felt that the microscopic picture was not conclusive.

During the following week the tissues in the region of the left mandible became inflamed, the second and third molars on that side loosened and were extracted. A small incision was made above the angle of the mandible and about 15 cc. of very foul pus evacuated, from which an anaerobic streptococcus was cultured.

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OSTEOSCLEROTIC ANEMIA SECONDARY TO EPIDERMOID CARCINOMA *

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Anemia is so frequently associated with carcinoma that we accept it as a concomitant feature, rather than showing surprise at its appearance. This anemia, however, as emphasized by Harrington and Kennedy,¹ and others, is almost invariably of the secondary type. von Grawitz² in 1879 described three cases of "malignant osteomyelitis" in which the blood pictures simulated that of pernicious anemia. Since that time a number of cases, more or less similar, have been reported and MacCallum,³ in classifying the anemias, speaks of this unusual type due to destruction of the bone marrow by tumor growth as "osteosclerotic anemia." The following case is one of this type that recently came under our observation.

CASE REPORT

Clinical History. G. S., a farmer 52 years old, complaining of pain over both thighs, arms, shoulders, chest and neck, and a fracture of the right femur, was referred to the medical service of the University of California hospital on Oct. 28, 1922, by Dr. Seawell of Healdsburg.† The family history is negative, but his past history is of interest, in that at the age of 14, when firing a rifle the ejected shell struck him over the left malar bone causing some injury to the skin. At the age of 32 he again injured in some manner the skin in the same area. This was followed by a chronic ulceration which lasted until four years ago when he went to a local "Cancer Institute" for treatment. The condition was diagnosed rodent ulcer and treated with arsenic paste: it cleared up with no subsequent local recurrence.

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† For the clinical notes we are indebted to Dr. N. C. Moffitt, Dr. W. J. Kerr and their associates.

On November 17, thrombosis developed in the right femoral vein which resulted in a marked edema spreading up into the perineum and right buttock.

On November 23, a splenic puncture was made. The smear from this was chiefly blood cells resembling the picture of pernicious anemia, with marked poikilocytosis and anisocytosis of the red corpuscles, megalocytosis and many normoblasts and myelocytes. In addition there were many abnormal cells about the size of small lymphocytes, each with a heavily stained nucleus and a basophilic cytoplasm containing from seven to nine chromotoid bodies.

On November 26, the right superficial epigastric vein became greatly distended with the blood flow directed cephalad. The abdomen, especially the lower portion, felt doughy, and the findings suggested a block of the inferior vena cava.

During his month's stay in the hospital, the patient's condition became progressively worse; the brownish pigmentation of the skin deepened and, during the last three weeks of life, the tumor in the left upper quadrant gradually decreased in size to three-fourths of its size on entrance, resembled the consistency of tense muscle and presented a smooth sharp edge that could be definitely followed around from midline to flank. Later, this tumor mass was definitely diagnosed as spleen, but until this time hypernephroma, adrenal neurocytoma and plasma cell myeloma of the bones with splenic involvement and especially a primary tumor of the spleen had been considered.

The patient died at midnight November 29.

The necropsy, the positive findings of which are recorded below, was performed eleven hours later.

NECROPSY REPORT

A ventral incision shows pale, flabby musculature and a decrease of the fatty tissues which appears hyperchromatic.

Peritoneal Cavity: The liver edge extends 3 cm. below the ensiform cartilage, and the spleen which is covered over by omentum extends 6 cm. below the costal margin in the left midclavicular line. Distally from the third lumbar vertebra, the vena cava and bilaterally the common iliac veins with all their branches, including the hemorrhoidal and prostatic plexuses and the median sacral vein, are widely distended by a firm, dark red thrombus which has originated somewhere below the origin of the external iliac veins.

Thoracic Cavity: There are a few old fibrous pleural adhesions to the apex of the left lung, about 50 cc. of clear straw-colored fluid in the pericardial sac, and multiple fractures of the second to tenth ribs inclusive on the left side, and third to tenth on the right.

Heart: Weight 250 gm., shows moderate dilatation of the right auricle, an anatomically patent foramen ovale, fenestrations of all cusps of the aortic valve and of the median cusp of the pulmonary;

TABLE I
Blood Values

Date	Red blood cells	Hemoglobin	Color index	White blood cells	Differential	Abnormalities
10-28-22	<i>per c. mm.</i> 3,968,000	<i>per cent</i> 75 Sahli	.94	<i>per c. mm.</i> 6,700	<i>per cent</i> Polymorphonuclears 72	Slight poikilocytosis. Some central pallor. No nucleated redds. Reticulated redds 8 per cent.
11-7-22	2,816,000	50 Talquist	.88	4,200	Polymorphonuclears 70 Small Mononuclears 8 Large Mononuclears 22	Marked central pallor. Moderate anisocytosis and poikilocytosis. No nucleated redds.
11-16-22	2,592,000	50 Sahli	.96	6,400	Polymorphonuclears 70 Small Mononuclears 12 Large Mononuclears 6 Myelocytes 12	Marked central pallor. Moderate anisocytosis and poikilocytosis. One normoblast seen.
11-24-22	2,080,000	40 Talquist	.96	7,200	Polymorphonuclears 80 Small Mononuclears 8 Large Mononuclears 4 Myelocytes 8 Myelocytes 12	Moderate anisocytosis and poikilocytosis. Three normoblasts seen.
11-26-22	Blood smear only					Moderate poikilocytosis and anisocytosis. Embryonic cells of all types; giantoblasts, megaloblasts, normoblasts. Several nucleated redds with dividing nuclei, some pyknotic. Many megalocytes.

are small, discrete, freely movable and but slightly firmer than normal. The fresh surface has a diffuse chrome yellow color but shows no evidence of hemorrhage or necrosis.

Skeletal System: The cranium, all vertebrae and ribs, the left ilium and both femora show similar changes, and a description of the cranium will suffice for all. On reflecting the scalp, the pericranium was detached from the calvarium with the greatest ease because it was almost entirely separated from the latter by diffuse collections of clear, pale yellow, viscid fluid in which great numbers of discrete, irregular, firm, white, flattened sand-like particles varying up to 2 mm. in diameter are suspended. The surface of the skull is extensively eroded by broad, shallow, sharply chiseled out furrows which cover the entire surface in an interlacing, geographical pattern. The calvarium likewise separates from the dura with remarkable facility and the same condition is found between the calvarium and dura as between the calvarium and pericranium.

The ribs and femurs seem to be lying in distended tubes of thickened periosteum, and separated from the latter by the same type of viscid fluid and granular particles, which are described above. These fluid accumulations are most marked along the ribs and in the left iliac fossa. Under the periosteum of the left ilium there is a fluctuating tumor mass bulging out into the false pelvis, which contains about 150 cc. of viscid brown-stained fluid and many thick gelatinous clots. The periosteal surfaces of all the bones are diffusely roughened by small irregular depressions giving a granular appearance. These depressions are all filled with the characteristic fluid and, in the areas of greatest fluid accumulation, erosion of the bone surface is most marked. All the bones, but especially the ribs, are more pliable than normal, fracture very easily and may be cut with the knife or sawed with greater facility than normally; and the sawdust resembles that of wood, being soft, dry and flaky. The marrow from the femur is yellowish white, fibrous with scattered edematous or gelatinous areas, and with a diffuse distribution of fine spicules of bone. The marrow spaces of the ribs are likewise filled with firm yellowish white material and a few gelatinous areas. Neither red nor fatty marrow is found in any of the examined bones. The fracture of the right femur shows fibrous union with very slight callus formation, limited motion between the fragments and some rarefaction of their ends.

considerable atheromatous sclerosis about the base of the aorta, and cloudy swelling of the myocardium.

Right Lung: Crepitant throughout and has a smooth and glistening but slightly opaque pleura. Scattered over the surface of the lung but more numerous on the interlobar septa are a few small, firm, white, puckered areas which when sectioned are firm and almost cartilaginous and appear to be quite superficial. Some of these areas have pale yellowish centers. At the apex of the lung there is some puckering with increase in fibrous tissue and slight calcium deposition, but no evidence of recent tuberculosis. Elsewhere the lung shows nothing more than hypostatic congestion of the dependent portions.

Left Lung: A similar picture is seen except that there is also thrombosis of the median branch of the left pulmonary artery with a resultant small, firm, red infarct with brownish borders in the middle lobe.

Liver: A little paler and firmer than normal, and over the lower anterior surface of the right lobe are two small, white circular non-elevated areas about 3 mm. in diameter, and sharply demarcated from the liver parenchyma and surrounded by a slight zone of congestion. These are quite superficial but, on sectioning the liver, similar yellowish areas are scattered throughout, apparently bearing no relation to the liver lobules. The gall bladder is negative.

Spleen: Weight 750 gm., and symmetrically enlarged. It is uniformly firm, about normal in color and the rather opaque capsule is smooth everywhere except over an area on the anterior surface, where it is adherent to the omentum by recent adhesions into which there has been some hemorrhage. The rather firm pulp shows a fine diffuse grayish mottling and does not readily scrape away with the knife; the Malpighian corpuscles are less distinct than normal, and there is slight fibrous thickening of the trabeculae.

Kidneys: Weight 250 gm. Negative except for cloudy swelling in both, and a superficial cortical cyst in the lower pole of the right.

Prostate: Negative except for a small round white phlebolith in the periprostic vein over the right lobe.

Gastro-Intestinal Tract, Pancreas and Adrenals are essentially negative.

Lymph Nodes: The bronchial group are moderately anthracotic but appear normal in size and consistence. The mesenteric nodes

In none of the sections is either red or fatty marrow to be found. Extending through the marrow spaces between the bony trabeculae, strands of fibrous tissue are seen; young in some areas and rich in large cells, and in others, largely hyalinized containing but a few small, thin, deeply staining nuclei and representing a much older fibrosis. Interspersed throughout this fibrous background are found groups of tumor cells varying greatly in character. Some groups in the younger fibrous strands are round clusters of plump cells showing cornification and pearl formation, while others show an irregular separation of the cells making conspicuous many intercellular bridges. The arrangement of such cells suggests the appearance of the diffuse epithelial masses seen in adamantinomas. Within the older more or less hyalinized fibrous tissue there is an occasional clump of a few, small, densely packed, deeply staining tumor cells (Fig. 7).

The changes in the cortical bone are most marked in the ribs, where the cortex is largely replaced by irregular layers of osteoid tissue covered on either side by new fibrous tissue, containing various groups of tumor cells. The periosteum is greatly thickened and infiltrated with the tumor tissue which in some areas is seen as broad sheets that have either split longitudinally or undergone central necrosis and become more or less cystic. Invading the original periosteum are strands of tumor cells which are clustered about blood vessels. Many small hemorrhagic areas are found in the regions of more recent growth, especially subperiosteally, while areas of cystic, gelatinous and hyaline degeneration are more common in the older growth.

Spleen: The changes here are well shown in Fig. 8. Scattered throughout the pulp and largely replacing its normal constituents are many myelocytes both singly and in groups of three to six, which are predominately eosinophilic though many are neutrophilic and occasionally there are seen myelocytes with less abundant cytoplasm which contain but a few, relatively large basophilic granulations. In addition to the definite myelocytes there are cells which have scant, poorly outlined cytoplasm in which no granules are evident, and large round nuclei that show deeply staining chromatin about the periphery and a few thin strands of chromatin crossing the pale central portion. These cells are probably myeloblasts and frequently one or two of them are surrounded by a group of myelo-

The Cranial Contents: There is an increased amount of cerebrospinal fluid and an old pachymeningitis with a very fine network of new capillary blood vessels over the tentorium. With the exception of edema of the meninges, most marked over the frontal lobes, the brain is negative.

The microscopic examination of the lungs, bones and spleen is of particular interest.

MICROSCOPIC REPORT

Lungs: The small, firm, white areas which are scattered over the pleural surfaces, consist of irregular clumps of epithelial cells surrounded by a desmoplastic reaction and, though rather superficial, lie not within the pleura but in the underlying lung tissue. These cell clumps vary greatly in type. In some areas they consist of scattered cells with lightly staining, irregularly ovoid or spindle-shaped nuclei, surrounded by scant, pale cytoplasm which extends in spider-like threads in all directions to unite with similar protoplasmic threads from adjacent cells. Between these cells there is a non-staining or faintly eosinophilic matrix, while about and extending throughout such a clump will be strands of fibrous tissue. In other areas, which are apparently younger and where desmoplasia is less marked, the cells are more uniform in type with round or oval, lightly staining nuclei surrounded by a moderate amount of pale cytoplasm. In such areas the cells are arranged in compact groups which show a tendency toward cornification and pearl formation in their centers. Though intercellular bridges are present in such groups they are not as prominent as in the previously described type. In a few other areas, probably older, a different picture is seen. This consists of dense, more or less hyalinized fibrous tissue within which a few scattered irregular strands of tumor cells are found (Figs. 5 and 6).

Bones: Sections for microscopic study were taken from the sternum, ribs, stenocondral junctures, femurs and pelvic bones. All these sections show a more or less similar picture, characterized by a complete infiltration of the marrow spaces, and considerable infiltration of the periosteum by tumor tissue which is accompanied by an extreme desmoplastic reaction. Associated with this, is a condition analogous to osteoid fibrosis, a decalcification of bone leaving a hyaline-like matrix and, in some areas, actual bone erosion.

TABLE 2

Location of Primary Tumor	Total No. of Cases	Reported by
Stomach.....	9	Schleip, ⁶ Parmentier and Chabrol, ⁷ Harrington and Teacher, ⁸ Harrington and Kennedy, ¹ Ellermann, ⁹ Fresé, ¹⁰ Kurpjuweit. ¹¹
Breast.....	5	Epstein, ¹² Houston, ¹³ Ward, ¹⁴ Piney, ⁴ Hirschfeld. ¹⁵
Prostate.....	2	Miller, ⁵ Braun. ¹⁶
Esophagus.....	1	Reichmann. ¹⁷
Appendix.....	1	Schleip. ⁶
Rectum.....	1	Piney. ⁴
Gall bladder.....	1	Kurpjuweit. ¹¹
Uterus.....	1	Hirschfeld. ¹⁵
Lung (alveolar).....	1	Piney. ⁴
Lung (bronchial).....	1	Piney. ⁴
Cheek (epidermoid carcinoma) ?	1	Rusk and Miles (present case).

show a peculiar blood picture, the outstanding features of which are as follows:

1. A marked reduction in the erythrocyte count with a similar reduction in hemoglobin, resulting in a color index that is usually slightly under 1.
2. Anisocytosis and poikilocytosis of the erythrocytes.
3. The appearance of immature red cells, both normoblasts and megaloblasts, in the circulation.
4. The appearance of myelocytes and less frequently myeloblasts in the circulation.
5. Usually a slight leucocytosis due principally to an increase in the myeloid series of white cells though frequently there is a leukopenia.
6. Frequently a terminal reduction in the blood platelet count.

DISCUSSION

One of the first questions brought to mind by our case is, why were metastases so extensive to the bone marrow and so limited elsewhere? There was no evidence of lymph node involvement anywhere in the body or of visceral metastases, with the exception of several relatively small foci in the peripheral portions of the lung parenchyma. The old conception, advanced by von Grawitz, that bone marrow tumors were the result of a "cancerous diathesis" that

cytes. Though a few normoblasts are encountered in the pulp tissue it is more likely that they have been swept in with the circulation than that they are the result of erythropoiesis, because they do not occur in groups but only as isolated cells, and no cells resembling megaloblasts can be found. The lymphoid follicles are not prominent and are relatively far apart, due to the increase of pulp.

With the exception of the infarcted region of the left lung, slight general passive congestion and parenchymatous degeneration, there is nothing further of interest in the microscopic examination of the tissues. The nodular areas seen during the gross examination of the liver are areas of senile sclerosis of the liver parenchyma. There is no tumor tissue in any of the sections of lymph nodes.

Briefly summarizing the case, we have, in a 50 year old man, an epidermoid carcinoma, arising probably in the skin over the left malar region, giving rise to metastases to the lungs and to the bones, where the marrow has been practically obliterated by the carcinoma, resulting in multiple spontaneous fractures of the bones and a peculiar type of anemia associated with a myeloid reaction in the spleen.

Cases similar to the above with metastatic involvement of bone marrow by tumor growth resulting in a specific anemia, are of rather rare occurrence in the literature. Piney⁴ in 1922 collected ten published cases and added four of his own. Since then a case has been reported by Miller,⁵ and by reviewing the literature we have been able to collect a total of twenty-three cases of bone marrow metastases that showed the blood changes characterizing osteosclerotic anemia. The location of the primary tumor in each of the accepted cases and the names of the authors who report them are listed below.

In addition to this list, several cases have been reported that may belong in it; but because of insufficient blood pictures, lack of definite bone marrow involvement, or some other important factor they cannot be definitely grouped with the above. Of these, von Grawitz has reported three cases; Schleip,⁶ one, a jaw tumor; Kurpjuweit,¹¹ one, related to lymphosarcoma; Waldstein,¹⁸ one, probably chloroma; and Israel and Leyden¹⁹ have reported a possible case due to osteosarcoma.

All of the twenty-four cases, in addition to being cases of metastatic involvement of the skeletal system, are alike in that they

bones mentioned. In other words, the bony metastases tend to locate within red marrow.

If we accept von Recklinghausen's conception that bone metastases occur by way of the blood stream, the above statements may bear some relation to the possibility that such metastases are the result of favorable mechanical or chemical factors in the red marrow. von Recklinghausen showed, and Drinker²⁶ has more recently reviewed the subject, that within the red marrow the blood vessels break up into many wide and tortuous thin-walled channels which results in a marked slowing of the blood stream. If cancer cells were free in the blood stream, it would seem that the partial stasis there would give them an excellent opportunity to gain a foothold within the many crevice-like outpocketings of these vascular sinuses. Ewing²⁷ while considering other possibilities, believes that the particular susceptibility of some tissues to develop secondary tumors is explicable on the basis of mechanical peculiarities of the circulation in those tissues. In addition to stasis, von Recklinghausen believed that physical strain and thermal changes were also important factors in the location of the bone metastases but his arguments are not so convincing.

The third possible factor namely, that metastases are dependent upon cancer cell virulence versus tissue resistance, may seem rather difficult to accept. However, it is well known that the bones and bone marrow show relatively poor resistance to infections, thus, regarding the carcinoma cells as invading organisms, it is conceivable that a carcinoma of relatively low virulence, which would be readily overcome by most of the tissues, could, providing it gained entrance to the bone marrow, obtain a foothold there and develop in this area of lower resistance.

The route by which cancer cells arrive in the bone marrow has for many years been a subject of dispute. Piney champions von Recklinghausen's conception that the metastases are blood borne; while Sampson Handley²⁰ is the outstanding exponent of the theory of lymphatic permeation. That the usual lymph node and visceral metastases come by way of the lymph stream can be readily shown in nearly all necropsied cases of carcinoma, and as such is generally accepted by all. However, we have been unable to find a record of any work proving the existence of lymphatics in bone marrow. Piney, using very careful technic, attempted the injection of peri-

could occur in several parts of the body simultaneously, is now untenable. At present, metastases are regarded as carcinomatous emboli occurring in either the vascular or lymphatic channels⁴ or due to lymphatic permeation.²⁰ With this in mind we present three possibilities that might account for this condition:

1. There may be a predisposition of certain tissues to metastasize to the bone marrow.
2. Mechanical or chemical factors may be present in the bone marrow which favor the development of metastases there.
3. The bone marrow, unlike the other tissues, may not possess sufficient resistance to prevent the development of the metastases of a carcinoma of a certain virulence.

The first becomes a very convincing argument when we recall the frequency with which carcinomas of the thyroid, prostate and breast metastasize to bones. Paget²¹ was one of the first to note this, and records the fact that of twenty cases of carcinoma of the thyroid which came to necropsy, ten showed metastases to bones. Risley²² states that Fischer-Defag, studying a series of prostatic carcinomas, found that twenty-five per cent showed skeletal metastases. More recently Moore²³ reports that of 1600 cases of carcinoma of the stomach, studied at the Mayo clinic during a period of ten years, not one gave evidence of metastases to bone; while of sixty-five cases of metastatic malignancy of the bones which he studied roentgenologically, thirty-six were from the breast, eleven from the prostate, seven from the kidney, (hypernephromas?) and two from the thyroid.

Not only are some carcinomas more apt than others to metastasize to bones but, as Paget, von Recklinghausen²⁴ and many others have observed, these metastases occur most frequently in certain bones and in certain parts of these bones. The spine is most frequently involved and after this the bones of the pelvis, the femurs, the humerus, the ribs and the flat bones in general. In relation to the femur and humerus, Pfahler²⁵ has emphasized that the metastases occur in the heads of these bones. von Recklinghausen conclusively demonstrated that the earliest bone metastases are in the centers of the bones within the marrow cavities. If we recall the anatomy of bone marrow, we note that normally red marrow persists throughout adult life in the head of the femur and of the humerus, and generally throughout the marrow spaces of the other

for by the theory of lymphatic dissemination, so it would seem that this case furnished a very definite example of spreading by way of the blood stream.

With our case it has been impossible to determine the route of invasion. The tissues have undergone such extreme carcinomatous changes that we are unable to say whether the metastases came in with the blood stream, located in the marrow, and by extension involved the bone and then the periosteum; or whether they entered the periosteum by way of the lymphatics, and then extended through to the marrow; or possibly by some combination of the two processes. However, the complete absence of demonstrable lymph node or fascial plane involvement, the fact that in general the periosteal growths appeared younger than the marrow cavity metastases, and that frequently within the original periosteum strands of tumor cells could be seen extending like cords about capillaries, lead us to believe that in this case the carcinoma was carried to the bone marrow by the blood stream and that the bone and periosteum became involved by extension from these metastases.

Anemia is the result of either increased blood destruction, blood loss, or defective erythropoiesis or a combination of the two processes. In this case there is no appreciable evidence of blood loss or destruction, and it is apparent that here the trouble is primarily due to defective erythropoiesis. As to the mechanism by which such an effect is produced upon the red marrow, little can be ventured. It may be, that as Jack and Teacher²⁹ have suggested, this effect is the result of some specific influence inherent in the invading tumor cells, but from a survey of these cases, it would seem far more probable that it is due merely to a widespread destruction of the erythroblastic tissues by the invading growth and the accompanying desmoplasia. McMaster and Haessler³⁰ have shown that the spread of the red marrow in anemia is dependent upon the available hemoglobin. Since in this case there was no blood loss and therefore no reduction in the available hemoglobin, we find that the red marrow has spread extensively to new fields but that following close upon it, extensive metastases have developed which tend to destroy the red marrow thus throwing the burden of erythropoiesis upon relatively small amounts of tissue which, in their so-called "hurried ineffectiveness," liberate many immature and abnormal cells into the circulation.

Opposed to this view are a number of cases reported by Middle-

osteal lymphatics and found that the material would readily enter the periosteal lymphatics and pass from them through the bone and into endosteal channels, but he could not force the mass from these channels into the bone marrow. The main reasons for Handley's contention that the metastases are carried by way of the lymphatics, may be briefly summarized as follows:

1. The distal bones of the limbs are rarely invaded by metastases. He states that this is incompatible with the embolic idea, because these bones would be just as liable as others to invasion and accounts for this phenomenon by saying that the patient usually dies before the process of lymphatic permeation has extended to them.

2. The liability of a bone to invasion increases directly with its proximity to the primary focus.

3. The point of attack in the limb bones is usually in that portion of the bone lying nearest to the cutaneous surface, which would be expected if the bones were invaded from the lymphatic plexus of the deep fascia.

4. Bone metastases occur almost entirely in the areas of the body that are apt to be involved by metastases to the skin.

Piney meets Handley's statements by replying that suitable soil for metastases occurs only in the red marrow, and in the adult this is not to be found in the distal bones of the limbs. As to the relation between bone metastases and skin nodule formation, he calls attention to the fact that they rarely occur together, though either singly is quite common. In addition to the above, Piney describes and gives photographs demonstrating groups of carcinoma cells lying within endothelial-lined channels in the bone marrow. He admits that he was unable to find both carcinoma cells and erythrocytes in the same channel, but states that such a picture has been observed by Assmann and by Erbslöh.

In this connection, it is worth while to mention a case of scirrhus carcinoma of the right breast which has recently been reported by Beatson.²⁸ He made an unusually thorough examination of the skeletal system, and in addition to involvement of the vertebral column, ribs and skull, all of which may possibly be explicable on the basis of lymphatic permeation or lymph stream embolism, he found metastases in both radii, ulnae, tibiae, fibulae and in the metatarsal and phalangeal bones of the left foot. Handley admits metastases beyond the elbow and knee joints are difficult to account

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DESCRIPTION OF PLATES

PLATE 83

- FIG. 1. Metastasis in lung.
 FIG. 2. Metastasis in femur.

PLATE 84

- FIG. 3. Detail with pearl formation in femur.
 FIG. 4. Myelocytic infiltration in spleen.

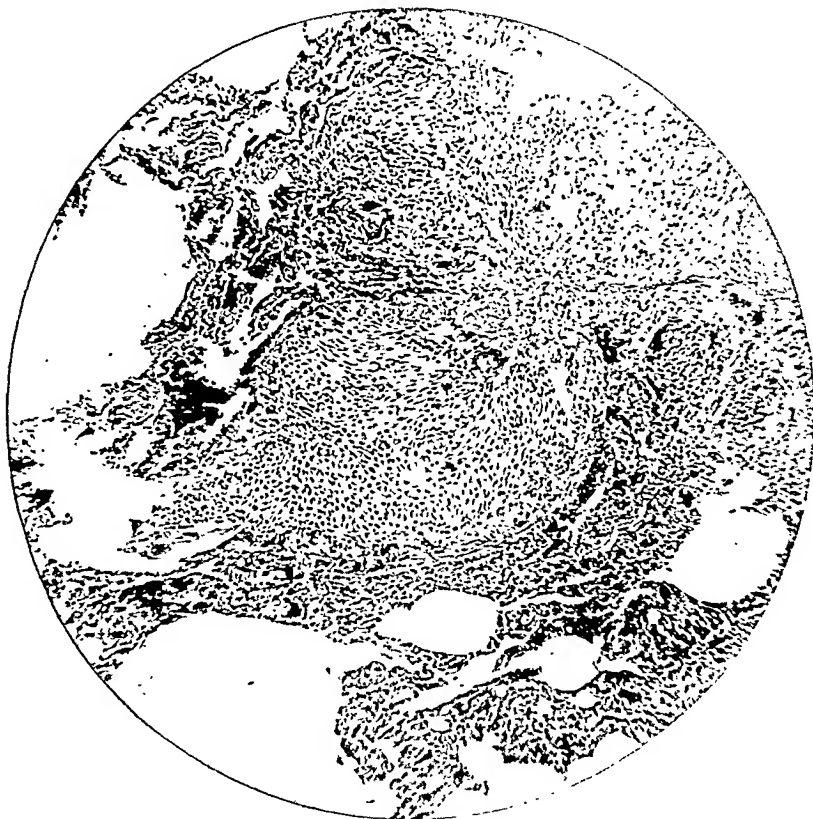
ton,³¹ Jack and Teacher, Thompson and Keiller,³² Beatson, and Nothnagel³³ in which extensive bone metastases were noted but which did not show an erythroblastic reaction. These can be explained by the above reasoning, only by assuming that in these cases metastases had not caused sufficient destruction of the red marrow to produce this picture before death ensued.

A feature of equal interest in this case is the marked myeloid reaction of the spleen. Though other men have mentioned the presence of a myeloid reaction of the spleen, Parmentier and Chabrol, Fresé and Kurpjuweit have made particular note of it. The cause of this reaction is to be attributed either to implants within the spleen of myeloid elements which have been carried by the blood stream from the bone marrow, or to a reversion of the spleen to its embryonic function in response to the destructive effect upon the myeloid elements in the marrow. Tendeloo³⁴ mentions both these possibilities but refrains from offering an opinion and merely states that it is an open question.

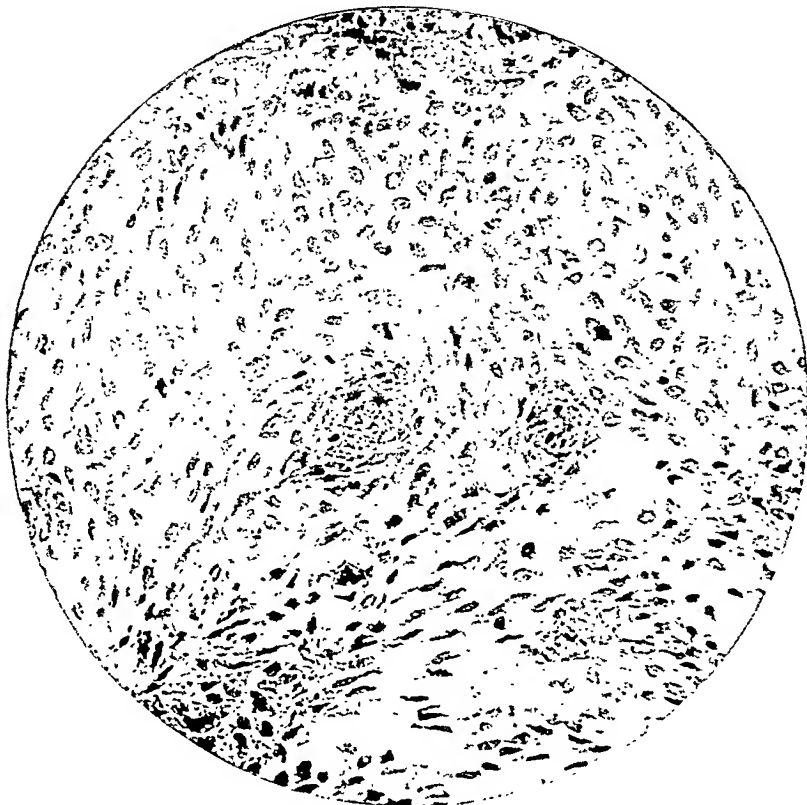
Considering our case, in regard to the literature which has been cited, we feel that the following conclusions are permissible:

CONCLUSIONS

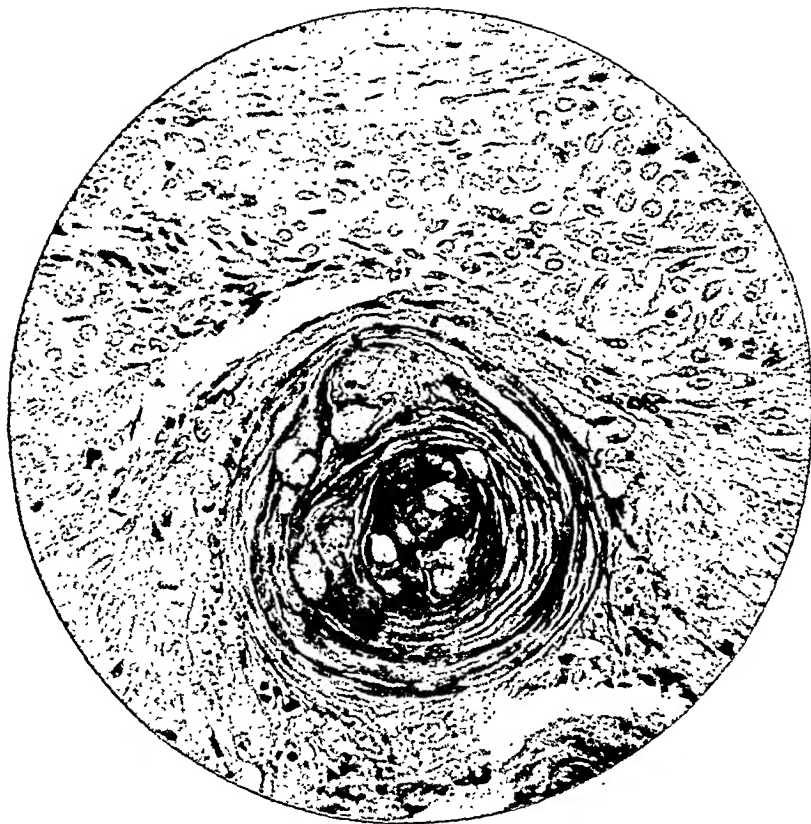
1. This case is one of epidermoid carcinoma probably arising in the skin over the left malar region and giving metastases to the lungs and to the skeletal system.
2. These metastases came by way of the blood stream and, in the case of the bones lodged first in the red marrow and then by extension involved the cortical bone and periosteum.
3. The picture here, which is primarily one of skeletal metastases, is most reasonably explained by assuming that carcinomatous emboli reached all the tissues but that only the bone marrow (excluding the lungs) furnished suitable soil and possessed the proper mechanical features to permit the development of metastatic tumor tissue.
4. The peculiar type of anemia produced is the result of widespread destruction of the marrow.
5. This anemia is characterized by the appearance of abnormal and immature erythrocytes, and myeloid leucocytes in the circulation.
6. With this anemia there is usually produced an extreme myeloid reaction on the part of the spleen, resulting in a splenic tumor.



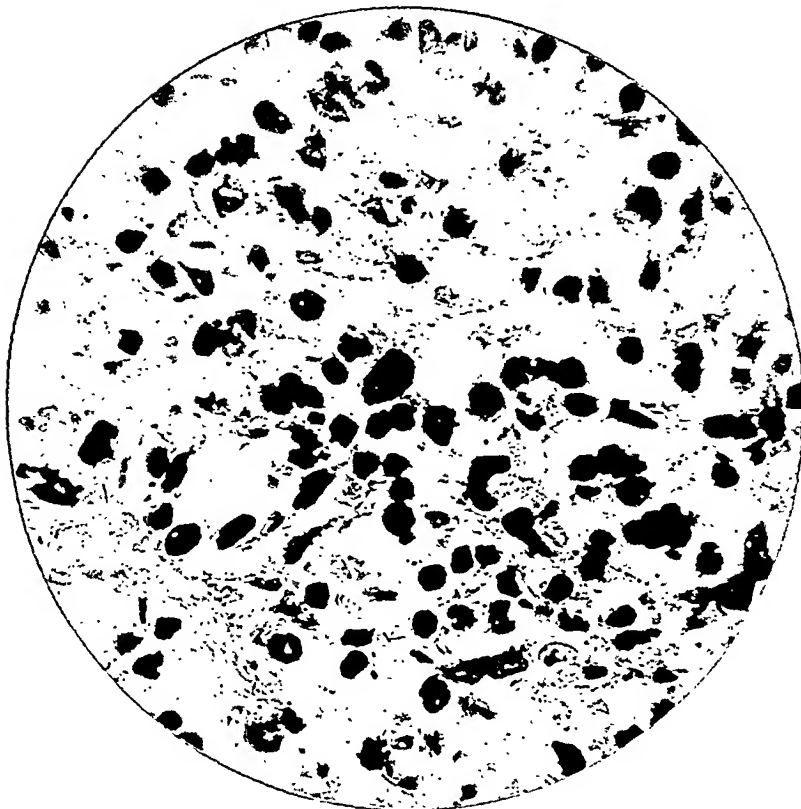
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of fungus infection reported below give a complete pathologic picture of the disease. The first case on the one hand represents the phase in which the parasite caused death in a relatively short space of time. The second case on the other hand represents the phase in which the host so successfully resisted the infection that clinically there was no suspicion that such an infection had ever existed. In both cases there are the intermediary lesions which interlock and thus a complete picture is procured.

The following is a brief summary of the clinical history and pathologic findings of the two cases.

CASE I. A white male of 19 years. His occupation was that of a common laborer. He had acne for a number of years. In January, 1926, the patient had a chest cold and a productive cough. Following this cold there appeared on the right side of the chest a sore spot which ulcerated and failed to heal. About the first of March the left knee became swollen and tender. March 30, the patient was admitted to the Wisconsin General Hospital for diagnosis and treatment of his knee condition.

On physical examination, granulating lesions on the right chest and left temple were found. The knee lesion was tentatively diagnosed tuberculous arthritis. No other physical findings of note were found.

Cultures of fluid aspirated from the knee and from the granulating lesions on the chest and temple yielded a fungus of undetermined type. Guinea-pig inoculations proved negative for tuberculosis. Biopsies from the skin and joint lesions showed the histopathology of a blastomycetic infection.

During hospitalization there developed a large number of subcutaneous lesions which ulcerated. Some of these healed while others did not. The right knee developed a lesion which by X-ray appeared to be tuberculous. The elbows, shoulder blades, skull bones and wrist joints developed similar lesions. The condition became gradually worse, resulting in death on Nov. 20, 1926.

The temperature ranged from 99 to 103 F. The white blood count varied from 13,000 to 30,000 per c. mm. with from 71 per cent to 83 per cent neutrophilic leucocytes.

Necropsy was performed 15 hours postmortem, only the body was examined.

Anatomic Diagnoses: 1. Generalized blastomycosis involving both lungs, both kidneys, lumbar vertebrae, numerous joints, bones and skin. 2. Bilateral iliopsoas abscess. 3. Acute pleuritis, right. 4. Congestion of liver and spleen. 5. Dilated right heart. 6. Marked emaciation.

The upper lobe of the right lung is firmly bound to the chest wall, is firm and on section presents the appearance of a caseous pneumonia with numerous small cavities. Scattered throughout the rest of the lung tissue on both sides are numerous firm nodules which on section resemble tuberculosis.

PULMONARY BLASTOMYCOSIS; ITS SIMILARITY TO TUBERCULOSIS. REPORT OF TWO CASES *

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Generalized fungus infections in man are sufficiently rare to warrant reporting, especially if cultures of the organism have been obtained. There is no doubt but that there is a considerable variety of fungi which cause lesions in the various tissues of man. It would seem that one of the most important pieces of work to be done in this type of infection would be for some individual worker to collect the various cultures that have been obtained and to work out their life history under conditions most suitable for their unhampered and natural development. No attempt will be made to discuss the nomenclature, cultural characteristics and classification of the organism obtained from one of the cases here reported. This organism, with several other fungi obtained from human lesions, is being studied by Dr. W. D. Stovall and will comprise the material for a future report. For those interested in the cultural characteristics, nomenclature and bibliography of fungus infections, the exhaustive work of Ricketts,¹ and Stoddard and Cutler² in this country are outstanding in their contribution to our knowledge of these diseases and their infectious agents. The first report of this type of infection in the United States was made by Rixford and Gilchrist³ in 1896.

The purpose of this report is to emphasize the similarity of the morbid anatomy and histopathology between the infections due to fungi and those due to tubercle bacilli. It would appear that the great variety of pathologic lesions produced in these two diseases depends largely upon the balance between the virulence of the infecting agent and the resistance of the infected host. For a clear conception of an infectious process of long duration it is essential to consider it from the very acute to the very chronic phase; and to compare infections caused by fungi and tubercle bacilli, it is logical that the different phases in one type of infection should be compared with corresponding phases in the other. The two cases

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muscular rheumatism. He worked in a large flour mill for 20 years, and for the past 12 years has been a church janitor. Otherwise his past history is negative, except for the usual childhood diseases. In December, 1925, he became acutely ill with a sense of pressure in the chest. He was seen by a physician who told him he had heart disease. On April 3, 1926, he was admitted to the Wisconsin General Hospital with an attack of cardiac decompensation. He remained in the hospital for a month and was discharged much improved. He returned to the hospital the latter part of June, 1926, in another attack of decompensation and remained until his death Dec. 11, 1926. The clinical diagnoses in the case were hypertension, arteriosclerosis, myocardial degeneration, cardiac hypertrophy, mitral and tricuspid insufficiency, chronic passive congestion of lungs, liver and spleen, fibrinous pleurisy and bronchopneumonia.

During hospitalization the patient showed a leucocytosis of 11,000 to 20,000 per c.mm. with 75 per cent to 90 per cent neutrophilic leucocytes.

Anatomic diagnoses: Generalized arteriosclerosis; coronary sclerosis; cardiac hypertrophy and dilatation; chronic valvular endocarditis; chronic fibrous myocarditis; chronic passive congestion of the viscera; anasarca; ascites; thrombosis of aorta; acute fibrinous pleuritis, right; acute gastritis; old tuberculosis involving both lungs and an occasional tubercle in the spleen and kidneys.

The only findings in this case which are pertinent to this report are those of the lungs, spleen and kidney, as the lesions in these organs were diagnosed as tuberculous.

The left lung is air-containing and shows the rusty appearance and hyperemia of chronic passive congestion. At the apex there is a definite puckered scar composed of numerous firm tubercles surrounded by pigment in the neighboring pleura.

The right lung has its pleural surface covered with a fibrinous exudate. It also presents the appearance of chronic passive congestion. A puckered apical scar is present and in the lower portion of the upper lobe and upper part of the lower lobe there are numerous firm pearly nodules in fan-shaped distribution which appear like old foci of tuberculosis. No cavity or caseous area involving vessels or bronchi is found.

Microscopic examination of the tissues confirms the above anatomic diagnoses except that the lesions taken to be tuberculous, prove to be due to a fungus. Numerous blocks of tissue were examined and they all showed the fungus present. Many sections were stained for tubercle bacilli, and careful and prolonged search failed to reveal them. The histologic picture of typical tubercles and areas of caseation are found. Giant cells, like those found in tuberculous

The lesions in the kidney are cortical and may not be distinguished grossly from tuberculosis.

Microscopic Diagnoses: The heart shows a small tubercle composed entirely of mononuclear leucocytes with three yeast-like bodies present.

The lungs show a variety of lesions. In the upper lobe of the right lung the alveoli are distended with an inflammatory exudate consisting of fibrin, neutrophilic leucocytes, mononuclear leucocytes and an occasional lymphocyte. Giant cells are very rare. Areas of caseation are present. Sections of the smaller nodules in other parts of the lung tissue show typical mononuclear tubercles, collections of neutrophilic leucocytes surrounded by mononuclear leucocytes and lymphocytes, and areas of caseation with mononuclear leucocytes and lymphocytes infiltrating the necrotic area. In these latter lesions giant cells, indistinguishable from those seen in tuberculosis are numerous. Yeast-like bodies are easily found in all these lesions, but in those showing caseation, "shells" of organisms are much more common than in the other types. In some of the sections there are irregular areas of fibrous tissue including a few giant cells, some of which contain yeast bodies in their cytoplasm.

Sections of the kidney lesions resemble those of the lung except that the fungi are more numerous, and giant cells are much more common.

A study of the fungus in the lesions shows no mycelia present. The organisms are all round or oval and have a double contoured refractile capsule. They reproduce by budding. No evidence of endosporulation is found. There is great variation in size of the organism, some of the giant bodies being three or four diameters larger than the smallest organisms.

The organism grew readily on suitable culture media. Branching septate hyphae, aërial hyphae with conidia formation and yeast-like bodies with refractile double contoured capsules, similar to the organisms present in the tissue, were produced in the cultures. It appeared that the type of culture medium, age of the culture and other conditions, such as moisture, largely controlled the gross and microscopic appearance of the fungus growth.

CASE II. The patient was a white male aged 60 years. When 6 years old he had a considerable number of large black and blue lumps on his lower extremities. At this time he had sore joints for three months. At the age of 56 he had

In previous publications^{7,8} I have submitted an interpretation of the histopathology of tuberculosis based on time sequence and on the balance established between the tubercle bacillus and the infected host. When interpreted on this basis the two cases of generalized blastomycosis here reported differ in no respect from tuberculosis in the pathologic lesions produced. The mononuclear leucocytes form tubercles and invade the caseous areas as they do in tuberculosis. The neutrophilic leucocytes participate in the formation of abscesses and in caseation as they do in tuberculosis. And the lymphocytes seem to appear at the same stages in both types of infection.

When stained with Sudan III there appeared to be just as great a lipid content in the caseous material of the fungus lesions as in the caseous areas of the tuberculous lesions. A point of interest here is that the living fungi had numerous globules which had absorbed the Sudan III while the "shells" contained no lipid material.

The production of giant cells appears to be analogous in the two types of infection. In Case I where there was little in the way of a reparative process, there were few giant cells; while in the second case, they were numerous. In other words it appears that these cells occur in the reparative stage of fungus infection just as they do in the tuberculous lesions. Many of the giant cells showed mononuclear leucocytes stretched out in the process of entering the area. Several giant cells were found which contained few to many particles of anthracotic pigment in the area about the nuclei, while the central area, which I have interpreted as an area of necrotic tissue in the giant cells of tuberculosis, was devoid of any pigment. In the adjacent areas individual mononuclear leucocytes containing pigment were commonly seen. The giant cells often contained bits of reticulum just as was found in the same structures in the tuberculous lesions.

There was great variation in the fungus content of the giant cells. In many of these cells no parasites were found, while in others as many as fifteen or twenty were present. Very commonly only the "shells" of the fungus were present, but in some instances living forms which often showed a budding process, were found. The distribution of the yeast-like bodies in the giant cells varied greatly. In some instances they were in the central area but more commonly they were in the nuclear zone at the periphery. In Case II, there

lesions, are numerous. Many of the lesions show extensive fibrosis with "shells" of the fungus lying among the strands of fibrous tissue, and no inflammatory cellular exudate is present. Several of the old fibrosed lesions show anthracotic pigment scattered throughout, a condition commonly seen in fibrosed tuberculous lesions in anthracotic lungs.

The fungus is a round or oval body with a double contoured refractile capsule. These bodies are very numerous in many of the lesions and few in others. In some of the smaller tubercles only one fungus is found in several sections. The large majority of the parasites are hollow "shells." In fact no parasite is found which has a reticular blue-staining cytoplasm similar to the typical staining reaction observed in the lesions of Case I. No culture was made of these lesions as their true nature was unsuspected at the necropsy. It is doubtful whether a growth would have been obtained for the parasites all appear to be dead.

DISCUSSION

In nearly all the reports of fungus infections with yeast-like bodies where the histopathology of the lesions has been studied, mention is made of the occurrence of tuberculous-like lesions. Gilchrist and Stokes⁴ state that "in a number of sections there was some evidence of the formation of tubercle-like nodules in the deeper portion of the chorion." Ricketts¹ in his excellent article notes that "the tubercloid nodules and multinuclear giant cells of cutaneous oidiomycosis possess few features to distinguish them from those of tuberculosis." Hektoen⁵ states, in speaking of oidiomycosis, that "real caseation and wholly typical tubercles do not seem to occur," while in coccidioidal granuloma, "it has been observed that this disease presents the best mimicry of tuberculosis ever seen and the lesions cannot be distinguished from tubercles by the microscope." Stoddard and Cutler² in their discussion remark that "we found in our cases and in our experimental work lesions so closely resembling tuberculosis that a differential diagnosis would be impossible without finding the parasites." Again, "the lesions have typical caseation or consist of miliary nodules without caseation." Davis⁶ found caseation in places and giant cells quite like tuberculous giant cells. Tubercles were also present but he thought they were not so clearly defined as in tuberculosis.

could more logically be explained on a hematogenous distribution than in any other way. Case II gave no history of any skin lesions.

Had an X-ray study been made of the pulmonary lesions in the cases here reported they probably would have revealed shadows indistinguishable from those seen in cases of pulmonary tuberculosis. Hence it would appear essential to bear in mind the possibility of a fungus infection in those cases where tubercle bacilli cannot be demonstrated in the sputum. This is especially important in those regions where fungus infections have been known to exist. This may also explain certain cases which clinically resemble tuberculosis, but in which tubercle bacilli cannot be demonstrated and in which the von Pirquet reaction is negative.

The gross and microscopic similarity of pulmonary fungus and tuberculous lesions would seem to necessitate the demonstration of the infectious agent before a positive diagnosis may be made. The difference in the pathologic picture, both gross and microscopic, appears to be one of degree only and not of absolute difference in the reaction of the body to the invader. In previous reports, I have called attention to the identity of the histopathologic lesions produced by the different types of tubercle bacilli. It would seem logical that fungi which are also closely related biologically would cause the production of identical lesions, if these fungi have the same degree of pathogenicity: that is, the more virulent the fungus the more acute is the inflammatory reaction, resulting in abscess formation; and on the other hand the less virulent the fungus the more chronic is the inflammatory reaction, as characterized by tubercle formation. Between these two extremes occur caseation and giant cell formation. The healed lesions appear identical.

CONCLUSIONS

1. Two cases of primary pulmonary blastomycosis are reported.
2. The identity of the gross and microscopic pathology in fungus and tuberculous infections is emphasized.
3. The necessity of bearing in mind the possibility of fungus infection as well as tuberculosis in the clinical and X-ray study of pulmonary lesions is suggested.
4. Before a positive diagnosis of tuberculosis can be established, the presence of the tubercle bacillus or the absence of a fungus

were colonies of "shells" of the fungus with many mononuclear leucocytes squeezed in between the individual parasites, but there was no demonstrable cytoplasm so commonly seen in the giant cell masses.

After a careful study of a large number of these giant cells it seemed that the fungus was not the real reason for their formation and that the inclusion of the parasite within the boundaries of such a structure was incidental or accidental. It would appear that the real force attracting the mononuclear leucocytes was a nidus of necrotic tissue especially difficult to dispose of, and that the presence or absence of fungi within the confines of the giant cell would depend upon whether these structures were within the area before necrosis took place. In other words it appears doubtful whether the giant cell, if regarded as an entity, has any power of phagocytosis. That the mononuclear leucocytes, whether fused or not, which wander to this area, may retain their phagocytic powers is not questioned. The presence of tubercle bacilli in the peripheral more commonly than in the central portion of giant cells would confirm the above interpretation.

A point of considerable interest in Case II was the presence of fibrosed lesions which contained dead fungi but no inflammatory exudate. It would hardly seem possible that so many parasites could grow in tissue without inflammatory cellular exudate, caseation and giant cell formation. And it would appear logical that after the parasites had been killed, the caseation and necrotic tissue had been removed by the mononuclear leucocytes and lymphocytes. During and following the removal of the dead tissue, fibrosis had occurred and the undigested "shells" of the parasites remained as inert foreign bodies between the fibrous strands. On this hypothesis, the presence of a solitary giant cell in the midst of a scar may be explained as being in an area in which the dead tissue had not been entirely removed.

The large size of the fungus makes its identification easy, and offers a clear interpretation of the reaction in the tissues in the acute and chronic phases.

From a study of the clinical histories in these two cases it would seem that the primary infection was pulmonary. Case I developed multiple skin lesions, but they were all subsequent to a definite pulmonary infection of some type. The distribution of these lesions

FIG. 4. A lesion in the pleura of Case II. Necrosis of tissue, mononuclear leucocytic and lymphocytic infiltration, giant cells and "shells" of fungi within and outside of giant cells. $\times 200$.

FIG. 5. A typical tubercle in the pleura of Case II. Note the giant cell in the center, and the fungus "shell" in the center of the area. $\times 200$.

PLATE 87

FIG. 6. Giant cell from lung of Case I. Note the empty "shells" among the nuclei of the giant cell at the right and above, and also lying free in caseous material in the lower left hand corner. In the upper left hand corner is an elongated mononuclear leucocyte. The black dots around the necrotic nidus of the giant cell are particles of anthracotic pigment. Note the anthracotic pigment in the individual mononuclear leucocytes in the lower right hand portion. $\times 400$.

FIG. 7. A giant cell similar to Fig. 8, but with viable fungi in the nuclear zone. The black dots are anthracotic pigment. $\times 400$.

FIG. 8. A giant cell in the edge of a caseous mass. From kidney of Case I. There is no anthracotic pigment. Note the dead fungi located as in the two preceding pictures. $\times 200$.

FIG. 9. A fibrosing tubercle in Case II with giant cells, and many dead fungi. $\times 200$.

PLATE 88

FIG. 10. A fibrosing tubercle in which there is very little dead tissue, and giant cells are wanting. Many dead fungi are present. $\times 200$.

FIG. 11. A portion of a completely fibrosed tubercle with many dead fungi, but with practically no inflammatory cells present. The numerous small dark dots are anthracotic pigment. $\times 400$.

PLATE 89

FIG. 12. Branching, septate mycelia growing in the depth of culture medium. Culture obtained from Case I. $\times 100$.

FIG. 13. Six weeks culture on blood serum. Natural size.

FIG. 14. Conidia on aërial hyphae from a month old culture. $\times 400$.

FIG. 15. Yeast-like bodies produced in culture. Such structures were more abundant on media such as blood agar, blood serum and potato, and were more numerous in old cultures. These structures resemble closely those seen in the pathologic lesions. $\times 400$.

should be demonstrated. The presence of the parasite and not the pathologic lesion will establish the type of infection.

I wish to express my appreciation to the orthopedic service and to the medical service of the Wisconsin General Hospital for the privilege of incorporating in this article a brief clinical abstract of the two cases reported. I also wish to thank Dr. C. H. Bunting for the privilege of reporting the necropsy and histopathologic findings in Case II, and for his constructive criticism in this study.

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DESCRIPTION OF PLATES

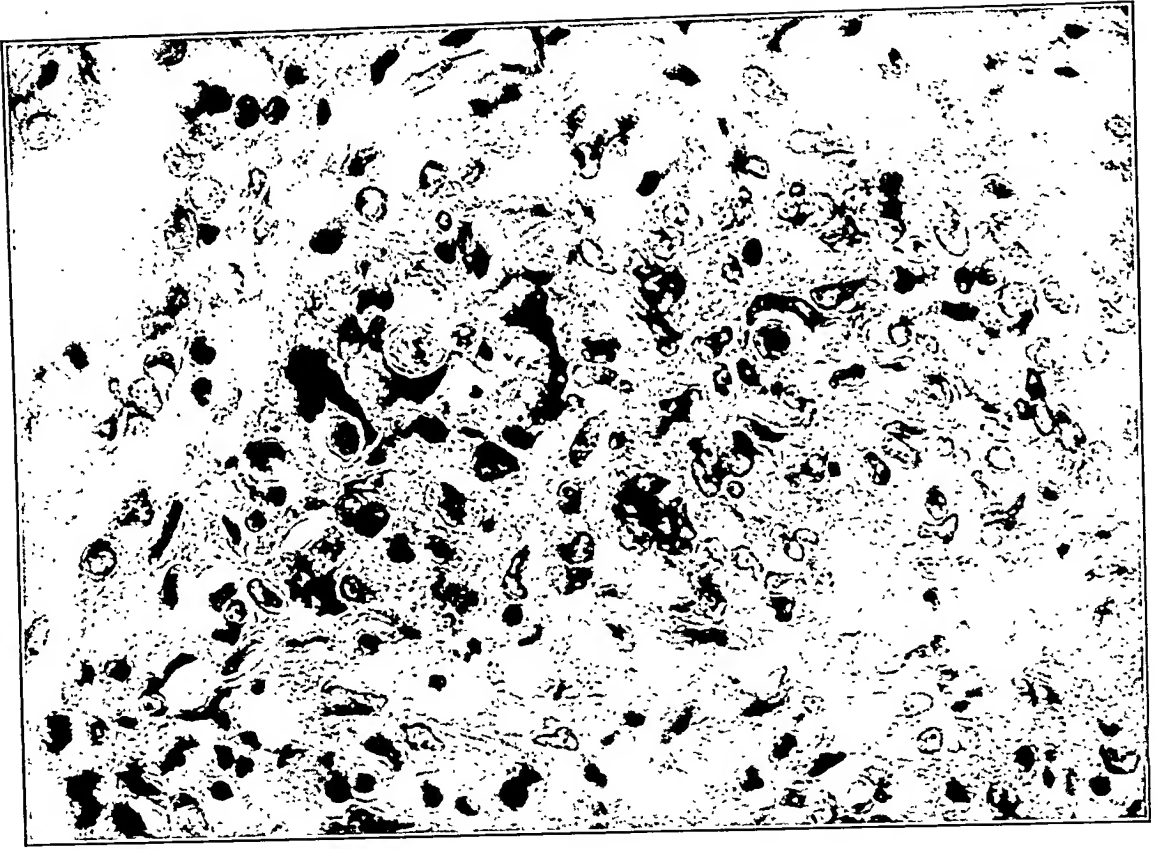
PLATE 85

FIG. 1. Early renal lesion in Case I. The inflammatory reaction consists almost entirely of mononuclear leucocytes. This resembles the early mononuclear tubercle of tuberculosis. There are several yeast-like bodies in the area. $\times 400$.

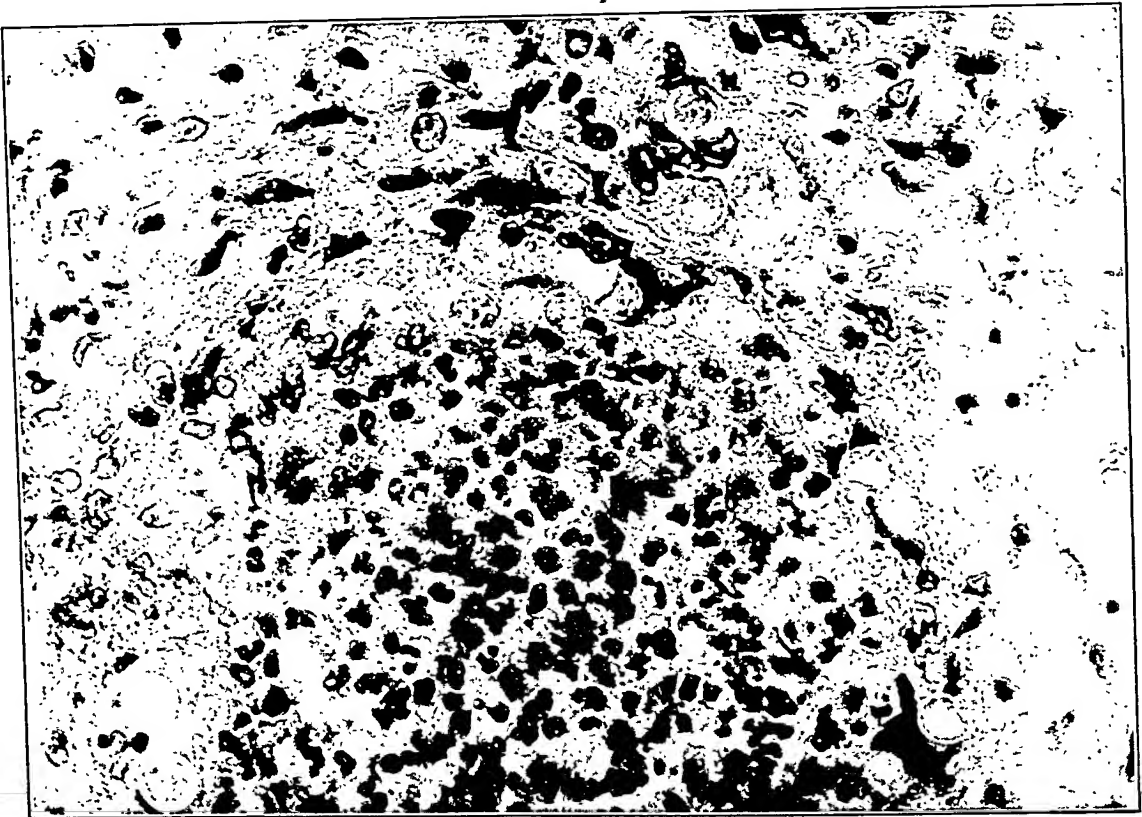
FIG. 2. An older lesion in the same kidney. The center of the lesion is heavily infiltrated with polymorphonuclear leucocytes. Microscopic abscess formation. Note the mononuclear leucocytes and yeast-like bodies at the periphery. There are also fungi in the abscess area. $\times 400$.

PLATE 86

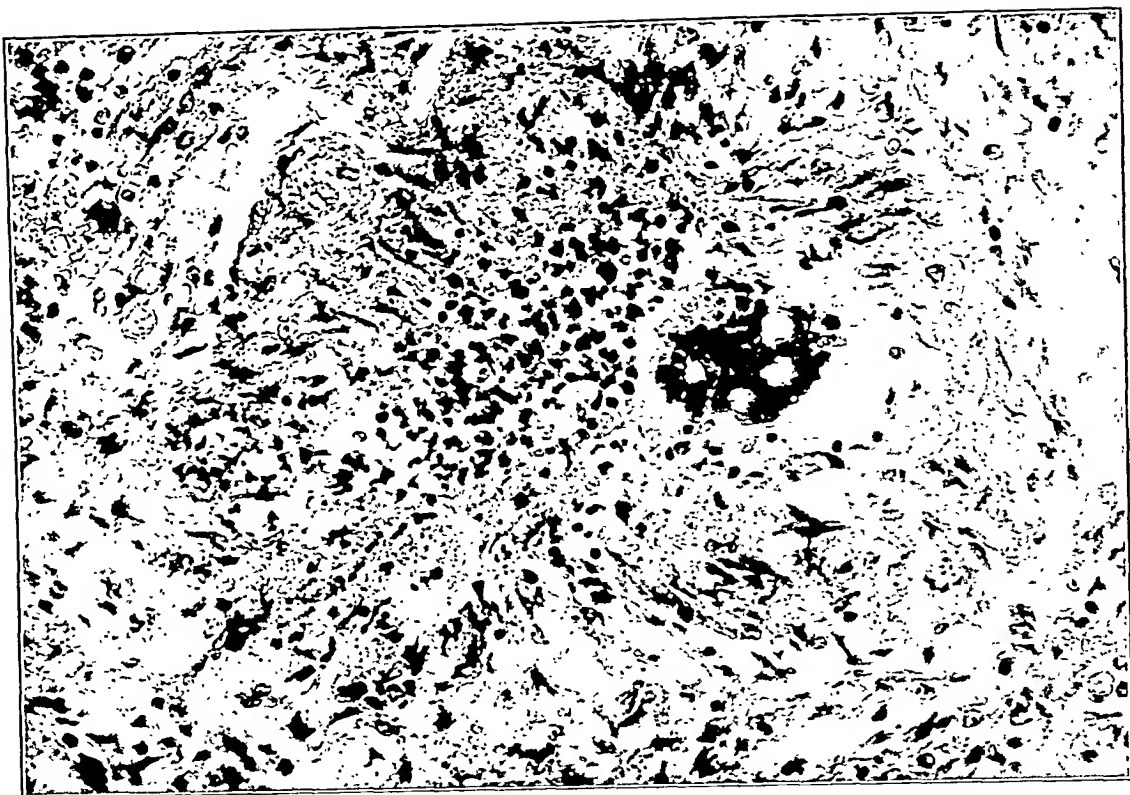
FIG. 3. A small caseating lesion in the lung of Case I. The central portion, composed of polymorphonuclear leucocytes, has not yet become caseous. Note the elongated mononuclear leucocytes and lymphocytes infiltrating the caseous periphery, the giant cell at the left and the absence of polymorphonuclear leucocytes about the periphery of the lesion. Most of the fungi in this lesion are empty "shells." $\times 200$.



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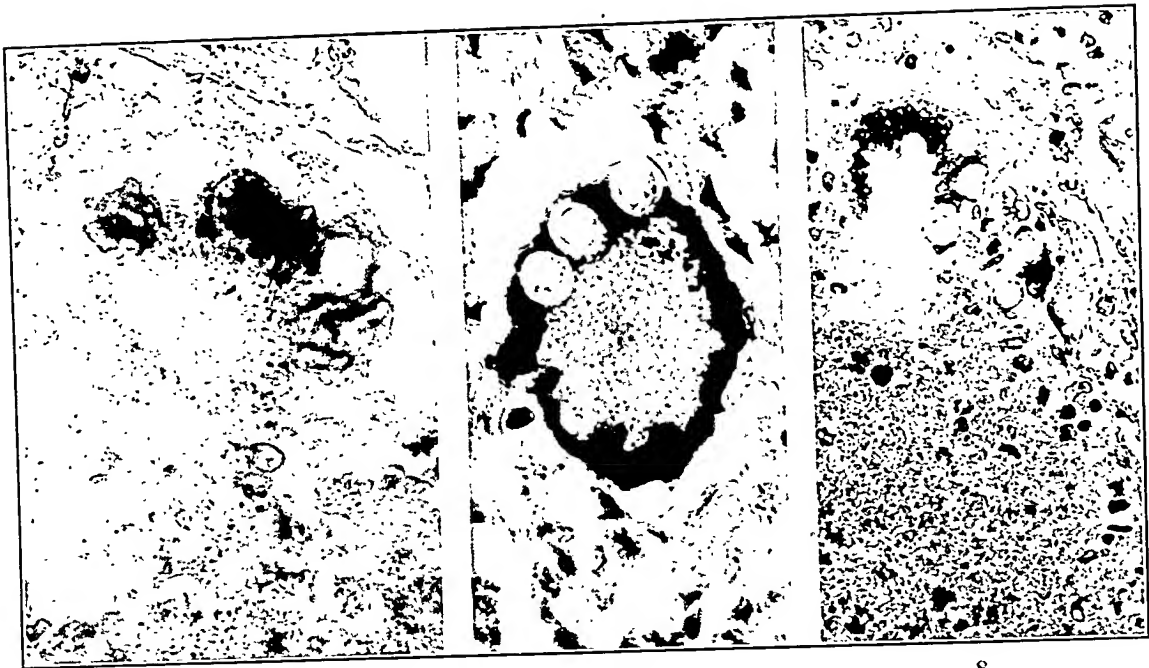
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Medlar



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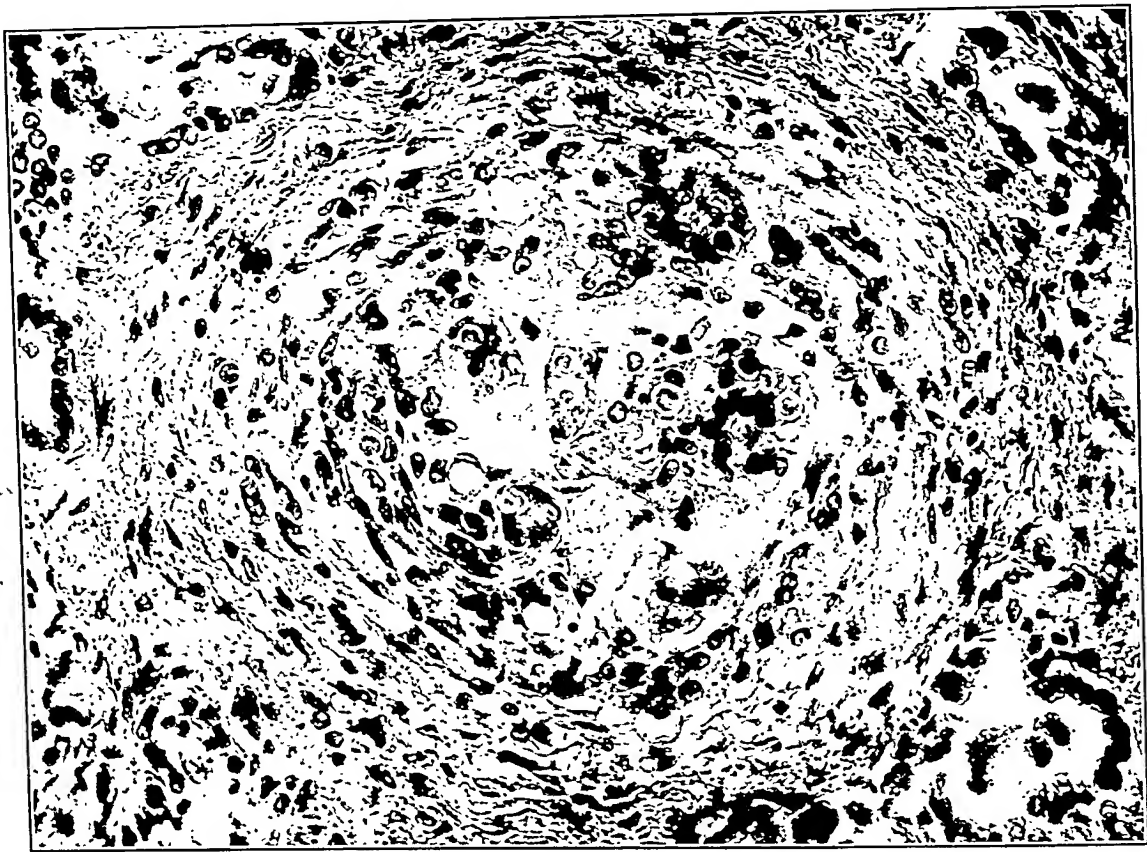
Pulmonary Blastomycosis



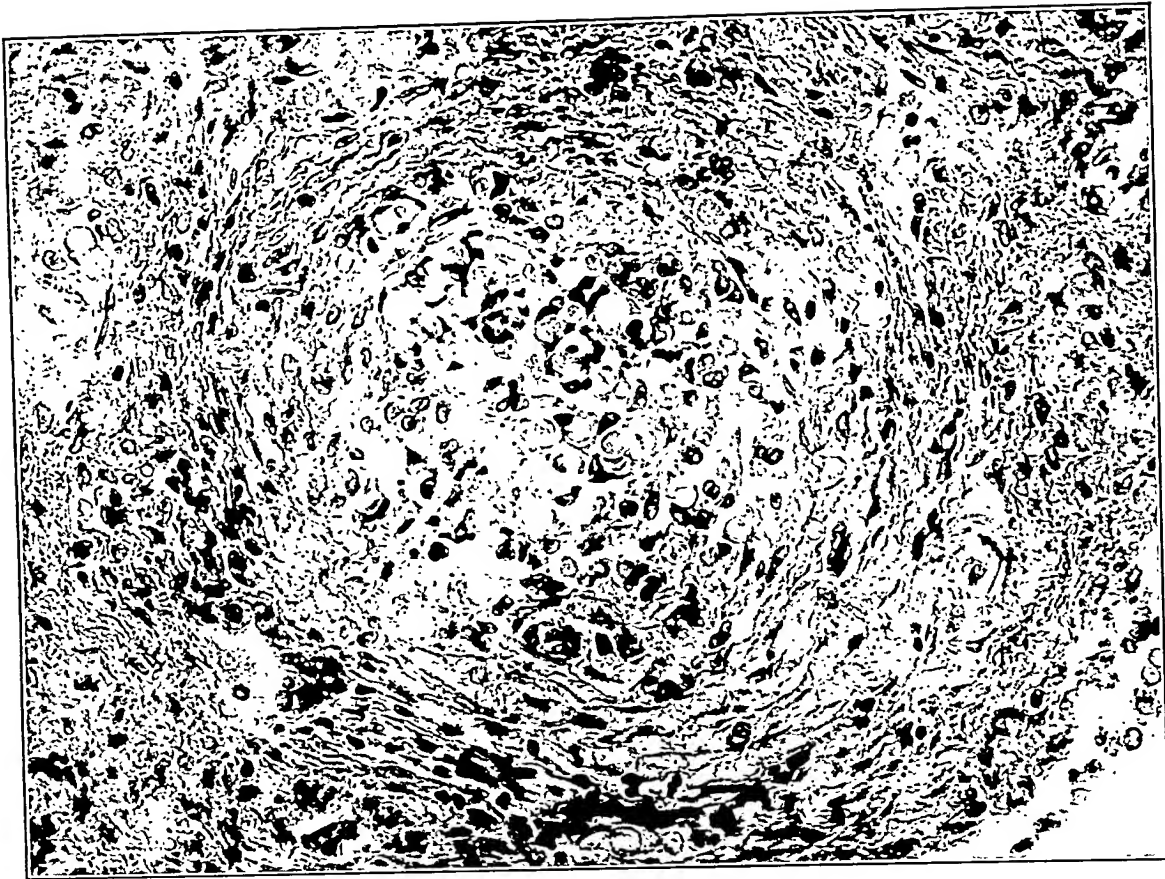
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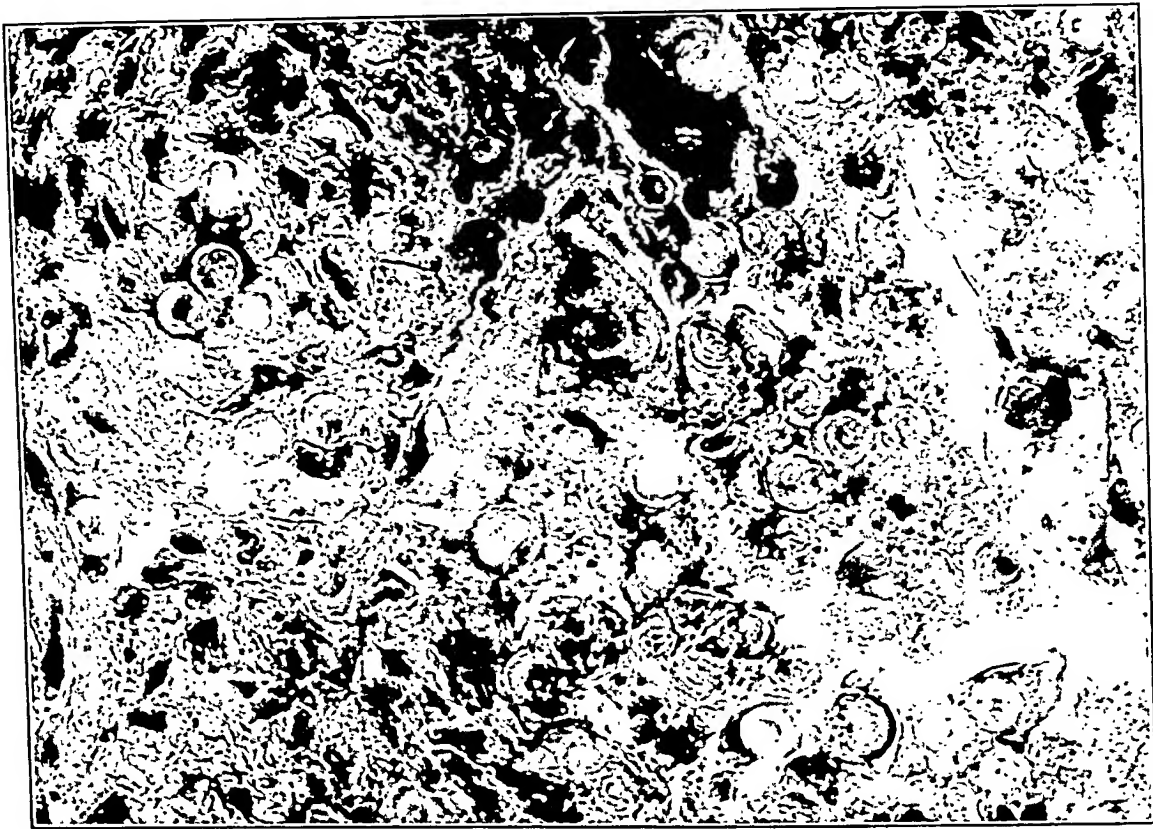
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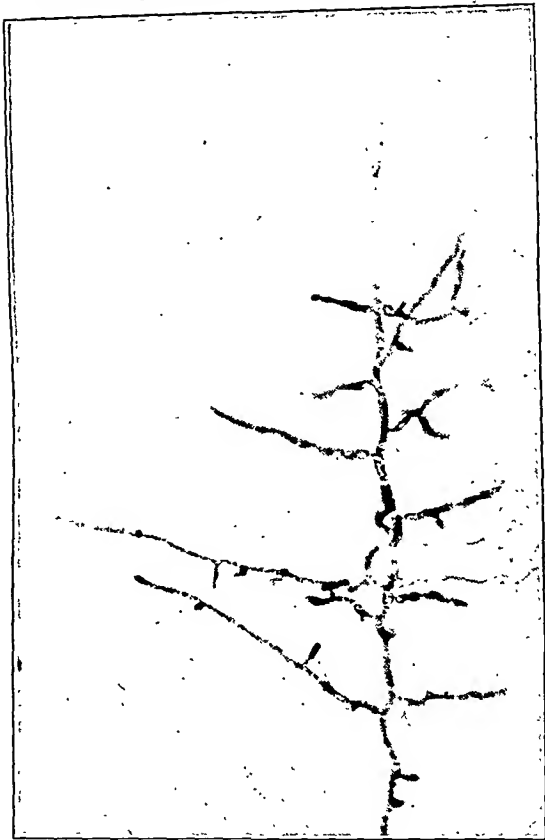
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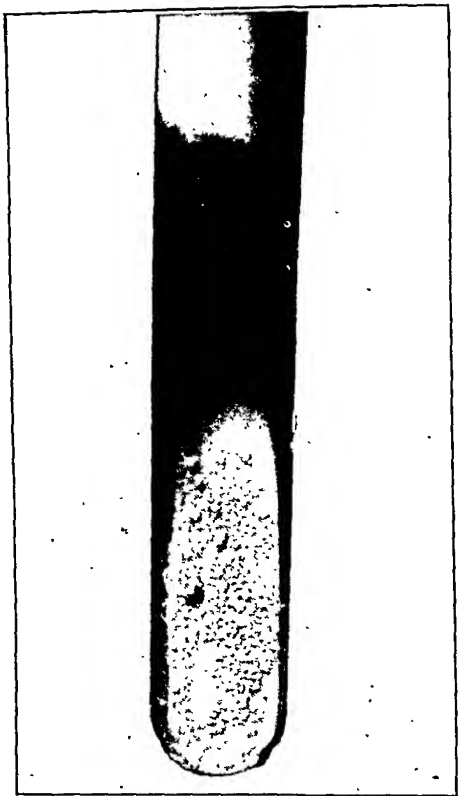
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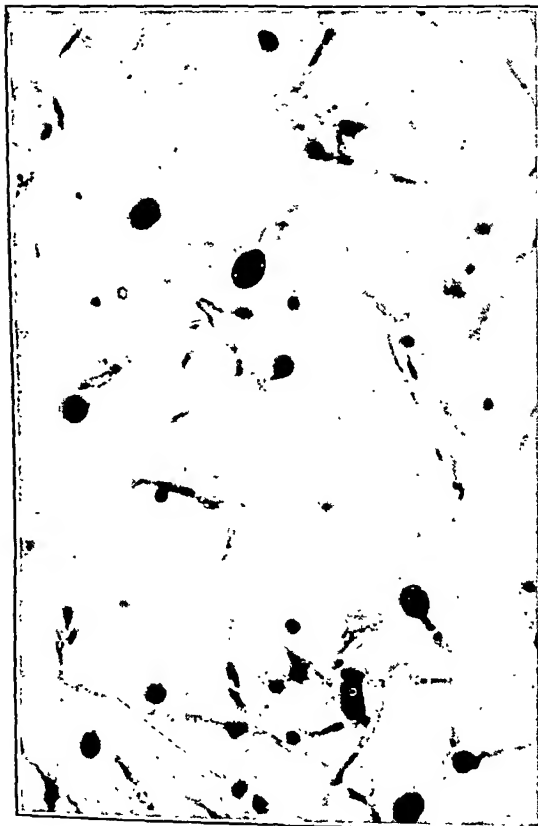
Pulmonary Blastomycosis



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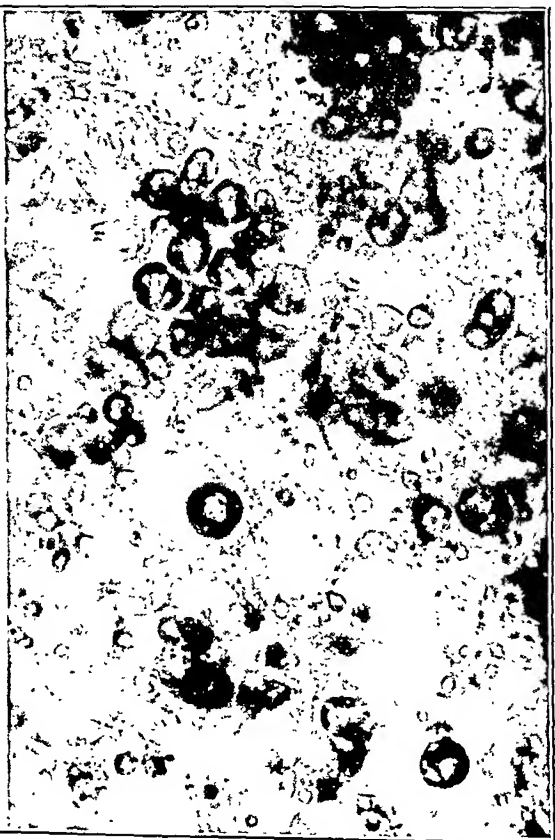


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Medlar



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Pulmonary Blastomycosis

cytes), lymphocytes, and a few polymorphonuclear leucocytes, which are enclosed within a dense network of reticulum. The reticulum in the walls of the alveoli is increased in quantity and is directly continuous with the network within the alveoli. Because of these characteristics which differentiate this type from the two preceding types, I have designated it *reticular pneumonic tuberculosis*.

If we compare a section of Type 2 (Fig. 1), of the above classification of bronchopneumonia, with a section of the lung in the case of acute blastomycosis (Fig. 2), it will be seen that, aside from the presence of the special organism of blastomycosis, they present the same characteristics, namely, a thickening of the walls of the alveoli, an increase in the amount of reticulum in their walls and no reticulum within the alveoli.

The exudate which occupies their lumina consists, in the majority of the alveoli, of mononuclear leucocytes, a few polymorphonuclear leucocytes and lymphocytes. Other alveoli contain numerous polymorphonuclear leucocytes and in some instances show the necrosis of early caseation. Whatever the character of the exudate may be, the organism of blastomycosis forms a prominent feature. Few giant cells are found. They may, or they may not, contain organisms, which when present, are usually arranged around the periphery of the giant cell.

In sections which have been impregnated by Bielschowsky's silver method the organism of blastomycosis is brought into special prominence, and this method gives a much better demonstration of the organism than staining with hematoxylin and eosin. Even in low power pictures the organism is easily recognized whether it be in the exudate of a bronchopneumonia (Fig. 2), or in a mass of caseation (Fig. 4).

When the living organism is studied under a high power (Fig. 3), the mode of propagation is seen to be by budding. On the other hand LeCount and Myers think that it is also propagated by means of spores, but I have not found them. The mature, viable organism is spherical and presents a characteristic stippled appearance; the same is true of the buds as soon as they have developed sufficiently to show a spherical outline, even though they are still connected with the parent organism.

In a previous contribution ⁵ I have described the part reticulum plays in the reparative process in miliary tuberculosis. It was there

THE RETICULUM OF THE LUNG *

V. ITS SIMILARITY IN BLASTOMYCOSIS TO THAT IN TUBERCULOSIS

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During the past year I have had the opportunity of studying the reticulum in two cases of blastomycosis which were placed at my disposal by my colleagues, Drs. Bunting and Medlar.³ One of these was a case of acute blastomycosis which occurred in a boy 18 years of age, and was correctly diagnosed during his life; the other was an unsuspected case of chronic blastomycosis in a man 62 years of age, and was not correctly diagnosed until sections of the lung had been subjected to microscopic study.

Blastomycosis, as it occurs in the human lung, has been described by Irons and Graham,¹ LeCount and Myers,² and others who have studied its pathology, as "essentially a bronchopneumonia and more a bronchitis than a pneumonia." In each of the cases studied by myself bronchopneumonia has been the outstanding lesion although, as LeCount and Myers have stated, many of the bronchi were almost obliterated and their position could only be determined by the accompanying blood vessels.

In an address given in New York before the Harvey Society,⁴ I described three types of bronchopneumonia that were associated with tuberculous lesions, and "each type presents a characteristic picture when stained for reticulum."

In Type 1, the walls of the alveoli are recognized with difficulty, and there is no increase of reticulum in their walls. No reticulum is found within the alveoli themselves. The exudate which fills the alveoli undergoes an early and rapid caseation. Miliary tubercles are rarely present.

In Type 2, the walls of the alveoli are increased in thickness and they contain an increased amount of reticulum. The alveoli are filled with an exudate in which caseation takes place quite early. Miliary tubercles are present in considerable numbers. The reticulum shows a tendency to encapsulate the caseous areas without however, extending into the alveoli themselves.

In Type 3, the walls of the alveoli are thickened; the alveoli themselves contain an exudate that consists largely of endothelial cells (mononuclear leuco-

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its further growth would probably cut off a segment of the caseous area and thus play an important part in the reparative process.

In Fig. 5, the caseous material has been still further absorbed and the lesion presents the characteristic picture of a miliary tubercle undergoing the reparative process. A large number of dead organisms are seen outside, but only a few within the caseous area. On the left, a small mass of caseous material has been separated from the main area by the extension of a band of reticulum which had, no doubt, an origin similar to the one described in Fig. 4. On the right side of the caseous area another band can be seen which, by its growth, would have divided the larger area transversely. Collagen has been deposited in the fibers around the periphery of the blastomycotic tubercle. In this figure the amount of caseation has been reduced while the reticulum has been correspondingly increased.

A still further reduction of the caseation and increase of the reticulum is shown in Fig. 6. The periphery of the blastomycotic tubercle is made up of collagenous fibers, and heavy strands are extending into the area of caseation. Many dead organisms are enclosed in the network of the collagenous fibers and of the reticulum. No giant cells are present in either Fig. 4, 5, or 6.

In Fig. 7, the only remains of caseation are a few necrotic cells in the center of the photomicrograph, and a giant cell in the upper right quadrant. Three organisms are included in the giant cell and they have the characteristic position on the periphery of the cell. Nearly all the fibers of reticulum have been converted into collagenous fibers. The organisms are not sharply outlined in the photomicrograph, due to the fact that the focus was centered on the network of fibers.

In Fig. 8, only a few remnants of the network of reticulum remain. The majority of the fibers are collagenous. All the organisms are dead and most of them show the characteristic hollow sphere; others present a broken outline, and a few have the "thimble-shape" sometimes assumed by the red blood corpuscles. The blastomycotic tubercle has passed through the entire reparative process.

shown that in the case of mononuclear or "epithelioid" tubercles, in which caseation has not taken place, there is a progressive development of reticulum which eventually is converted into collagenous tissue. In those cases in which caseation has taken place, unless the area of caseation be too large, the necrotic material is gradually absorbed and the reticulum eventually fills in the space thus created; the reparative process then proceeds as in the case of the mononuclear tubercle. The same process takes place in blastomycosis and it is possible to follow the changes in the organism, while in tuberculosis it is not so easy to demonstrate what happens to the bacilli.

In acute blastomycosis the active organism (Fig. 3), has a spherical shape and is marked with a pronounced stippling. With the death of the organism this stippling disappears and, in sections impregnated with Bielschowsky's silver stain, it appears as a shell with a clear center or as a uniform dark sphere (Fig. 8), depending on the plane which lies within the focus of the objective. In sections stained with hematoxylin and eosin, a homogeneous substance is often seen within this shell; possibly this may be the cause of the dark appearance of the shell above mentioned. In many instances a shell gives the impression of having been crushed when the section was cut; in other instances it presents an appearance not unlike the "thimble-shape" of a red blood corpuscle.

Taking up now the case of chronic blastomycosis, it was found that scattered mononuclear tubercles containing the organism were present, especially in the pleura; that numerous giant cells were present; that there was no acute inflammatory cellular exudate, and that the lesion resembled the reparative process in miliary tuberculosis.

In Fig. 4, there is an extensive area of caseation that is surrounded by a layer of collagenous fibers which are continuous with a network of reticulum that is growing into the caseous area. A large number of dead organisms are present outside the area of caseation and many organisms are also present within the caseous area although only a few of the latter appear to be living. It is evident that the progress of the caseation has been arrested and that a reparative process has already begun. On the upper border of the photomicrograph and a little to the left of the center, a band, partly of collagenous fibers, is beginning to extend into the area of caseation, and by

FIG. 6. A blastomycotic tubercle in which the area of caseation is smaller than in Fig. 5. Nearly all the fibers contain collagen. All the organisms are dead. Chronic blastomycosis. $\times 100$.

PLATE 93

FIG. 7. The only evidences of caseation in this figure are a few necrotic cells near its center and a giant cell on its right. The giant cell contains three dead organisms. There is still a small area in which reticulum is present; all the remaining fibers are collagenous. Chronic blastomycosis. $\times 400$.

FIG. 8. There is no evidence of caseation in this figure. All the organisms are dead. But few fibrils of reticulum remain; the rest are collagenous. Chronic blastomycosis. $\times 150$.

CONCLUSION

This study of the reticulum shows that its growth and transformation into collagenous tissue differ in no way in the tubercle of blastomycosis from that in the tubercle of tuberculosis.

If the presence of the special organism be eliminated from the picture, the lesion is the same in both infections.

This leads to the conclusion that, though the organisms differ, the lesion they produce cannot be differentiated.

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5. Miller, W. S. *Am. J. Path.*, 1927, iii, 217.

DESCRIPTION OF PLATES

All the sections were impregnated by Bielschowsky's silver method.

PLATE 90

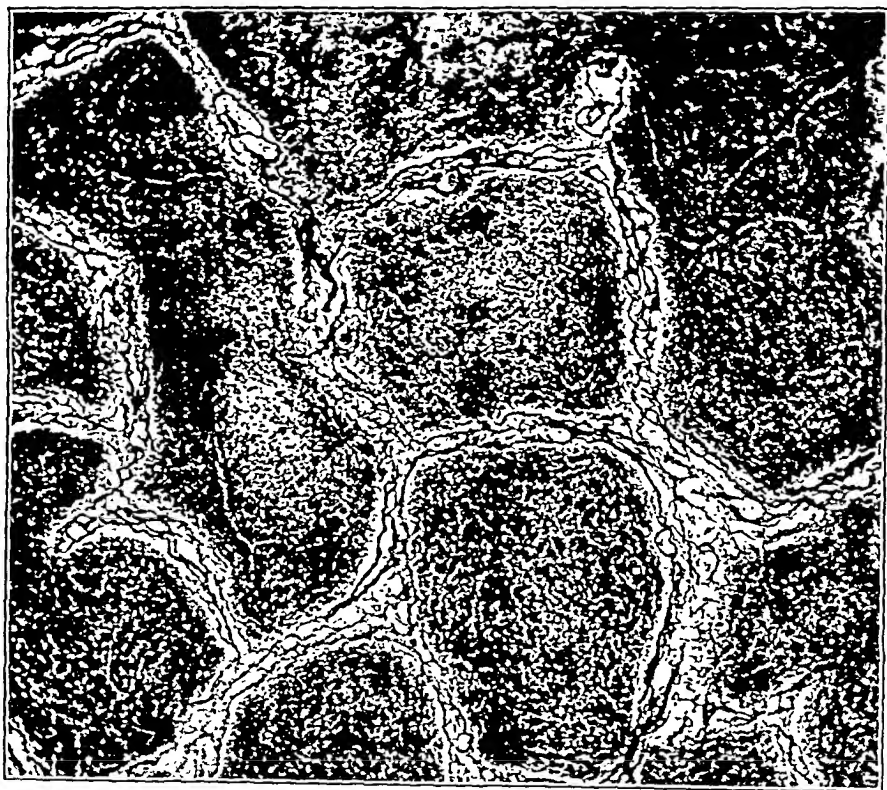
- FIG. 1. Type 2 of the tuberculous bronchopneumonia described in the text. $\times 200$.
- FIG. 2. The bronchopneumonia of acute blastomycosis. Same type as Fig. 1. $\times 200$.

PLATE 91

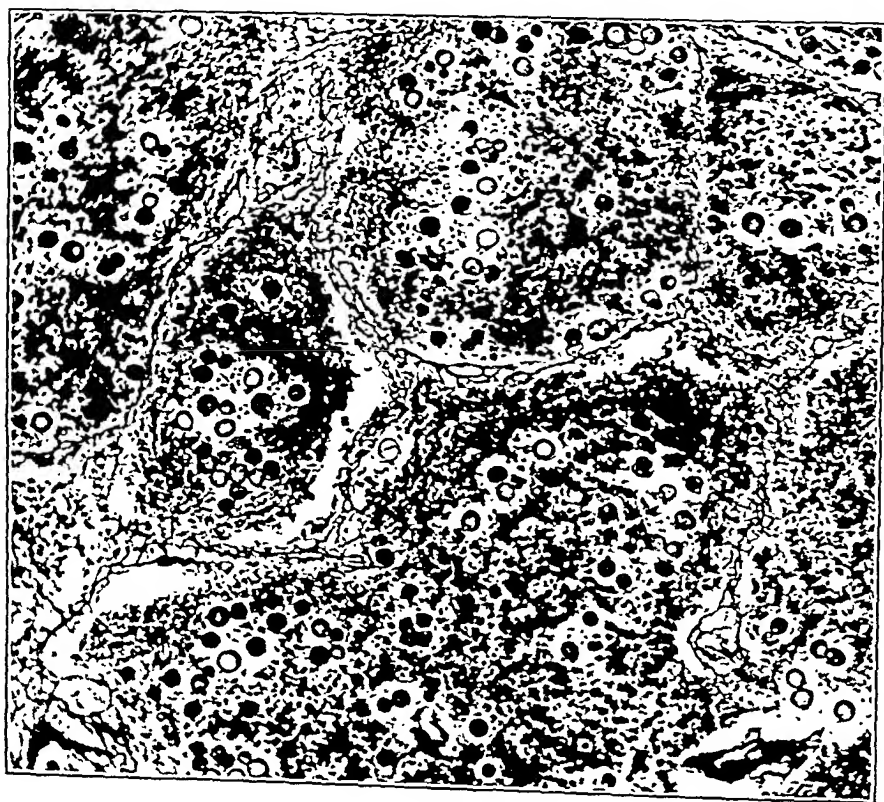
- FIG. 3. The organism of blastomycosis as seen in the exudate of acute bronchopneumonia in blastomycosis. Note the stippling of those organisms which are in exact focus; also the buds in various stages of development, and observe that they have the same markings as the adult cells. $\times 500$.
- FIG. 4. Part of an extensive area of caseation. The whole area was surrounded by collagenous fibers. Note their beginning extension into the area of caseation on the left side of the figure. Most of the organisms are dead; a few within the area of caseation are living. Chronic blastomycosis. $\times 100$.

PLATE 92

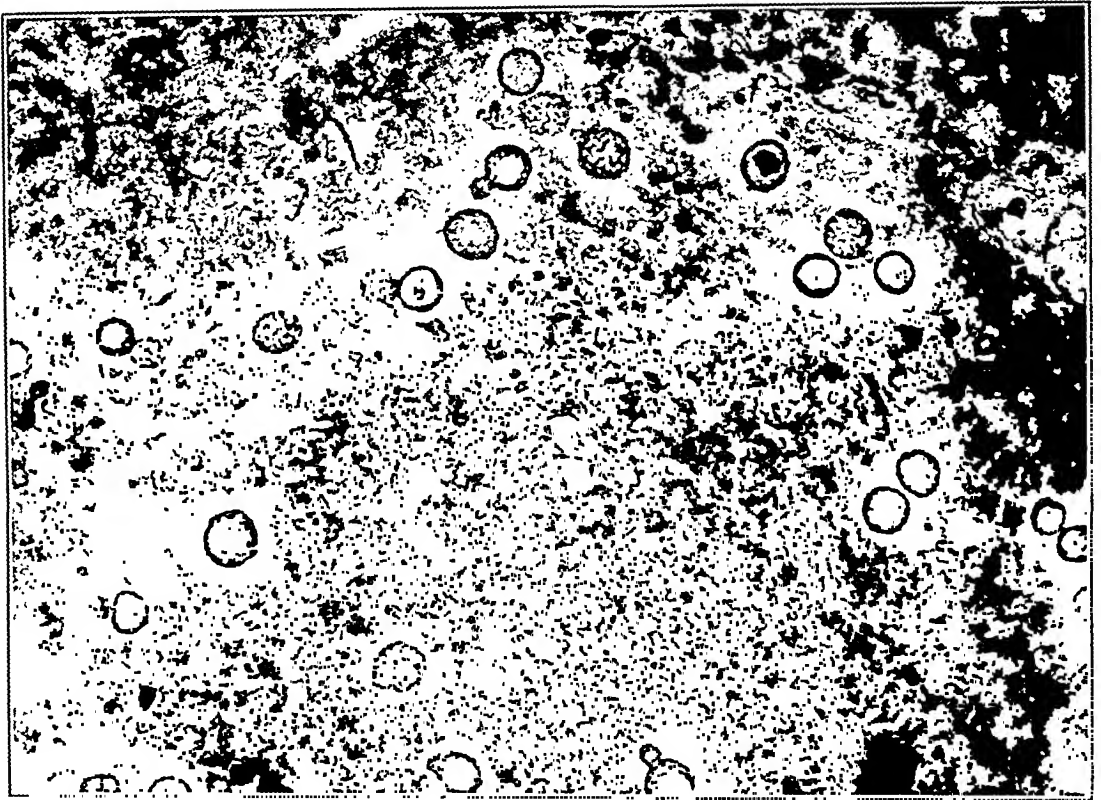
- FIG. 5. A blastomycotic tubercle in which absorption of the necrotic material is taking place. The area of caseation is being subdivided by the ingrowth of reticulum. All the organisms are dead. The fibers around the periphery of the blastomycotic tubercle and many of the fibers within the tubercle are collagenous. Chronic blastomycosis. $\times 100$.



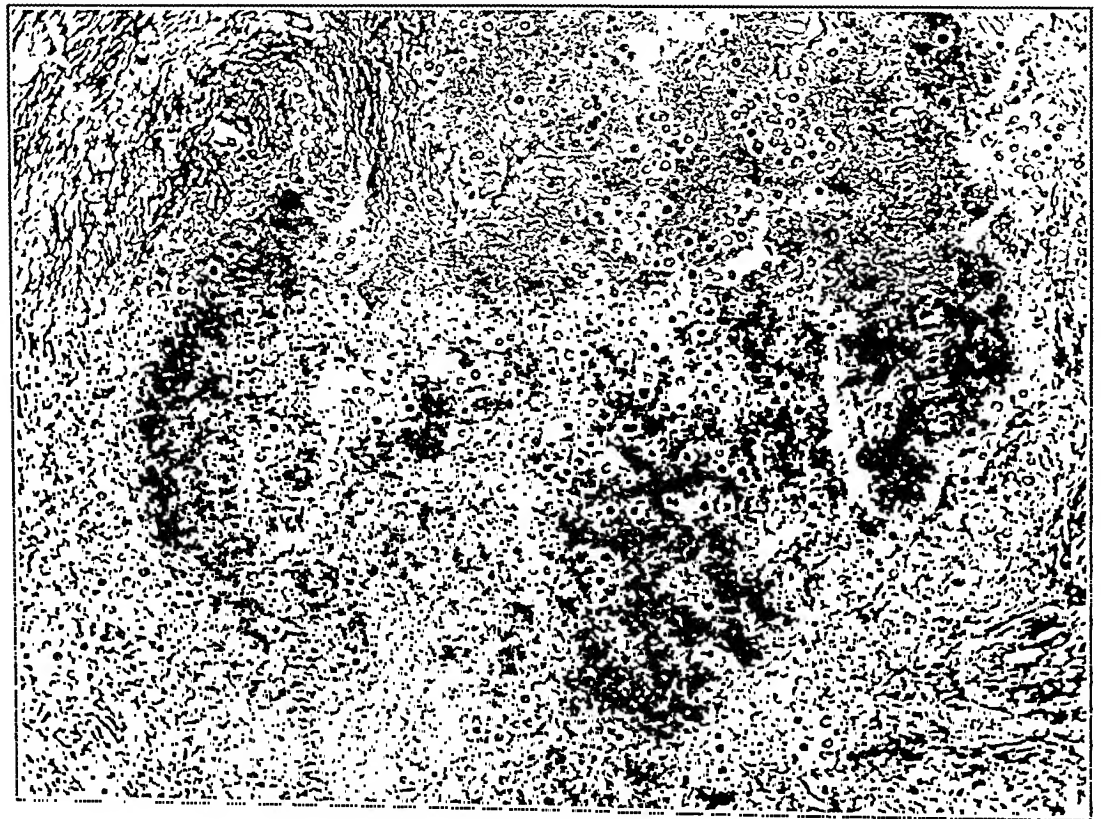
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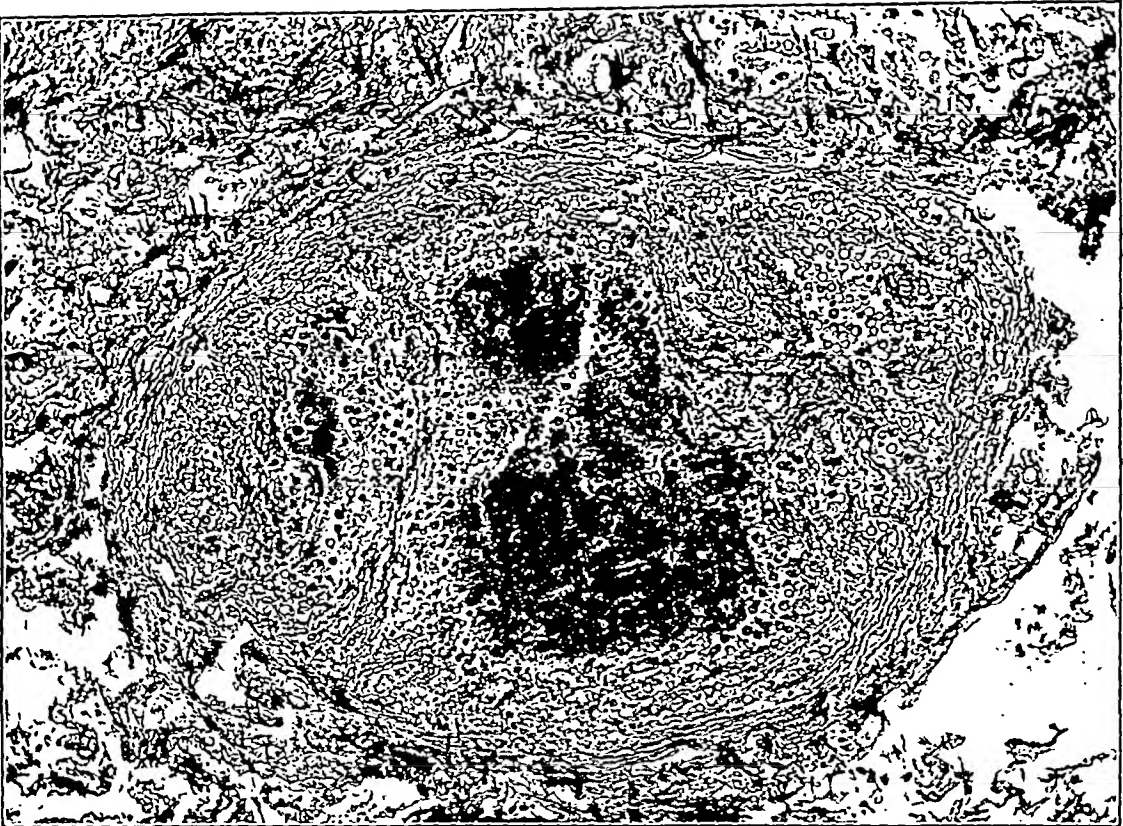
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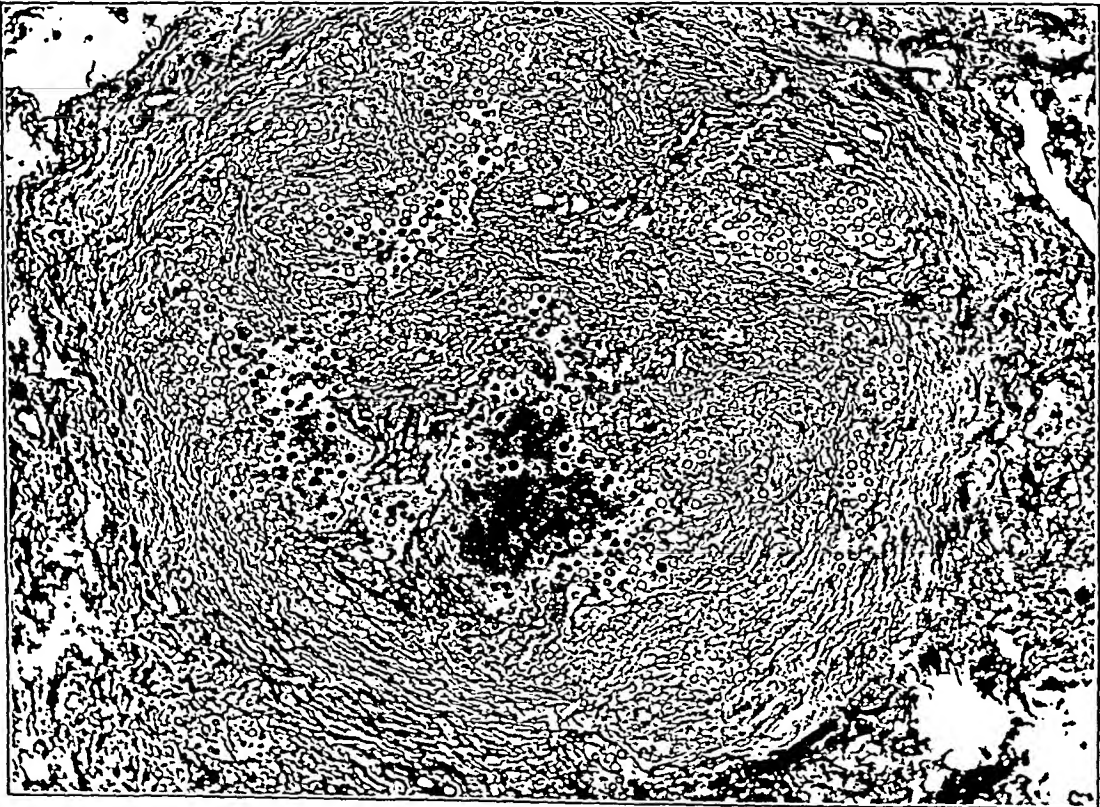
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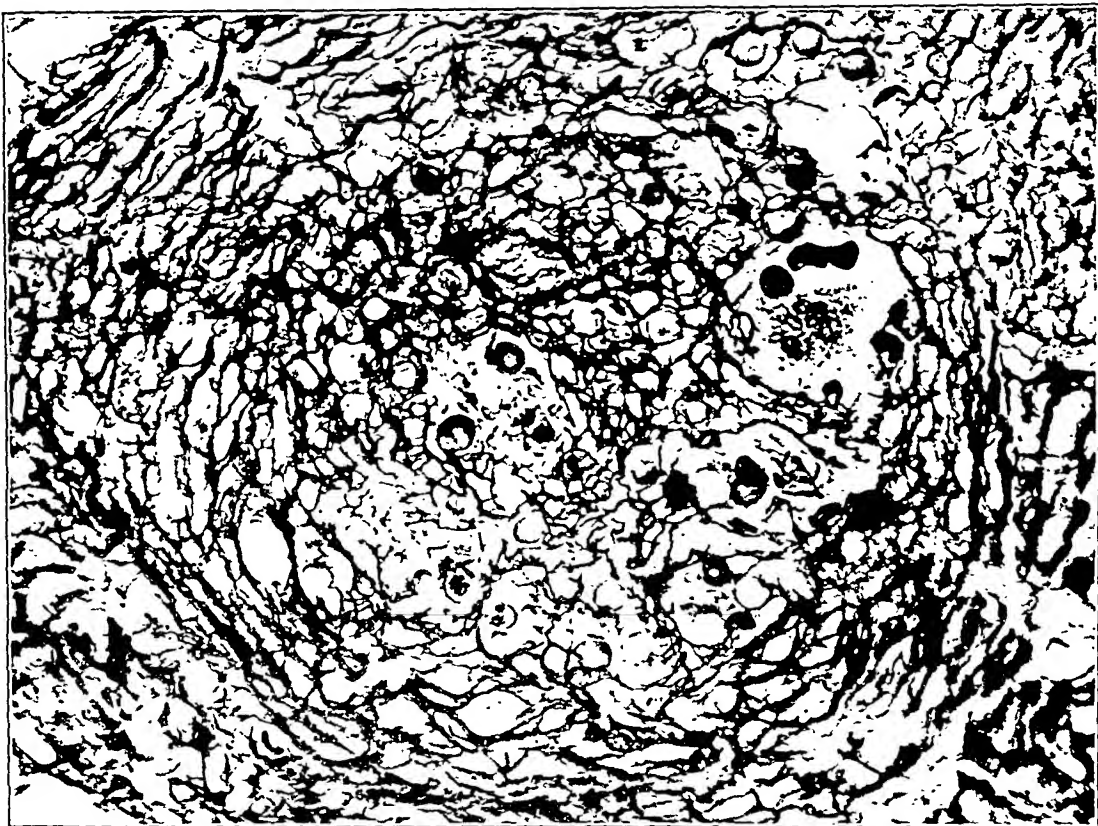
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Present Illness: Began during December, 1922, with itching and burning along the medial surface of the right leg. During January, 1923, a small nodule appeared in this area, just below the knee, which grew rather rapidly and was tender and painful at times. Later it ulcerated and bled very easily. He entered the Albany Hospital Jan. 25, 1923, and the tumor was removed under local anaesthesia. About six months later he noticed several more nodules on the medial and dorsal surfaces of the foot. These became very numerous and painful. Various ointments were applied until Sept. 9, 1924, when the patient re-entered the hospital for radium therapy. Two treatments of 500 milligram hours each were given. Since that time he has been at home and feels quite comfortable except that now and then some of the nodules become rather painful.

Physical Examination: An elderly male, up and about in his home. Pupils react to light and accommodation. Sight is slightly impaired due to bilateral cataracts. Conjunctivae of the lids are deep red. Slightly deaf in one ear. Nose is enlarged and shows marked reddening at tip due to many minute engorged blood vessels. Tongue appears normal. No teeth. Plates are used and give no trouble.

Chest is large and barrel-shaped. Considerable adipose tissue present. Apex beat is not visible or palpable. Cardiac dullness extends slightly beyond midclavicular line in fifth interspace on left side of sternum. Heart sounds are strong and regular except for an occasional extra systole. Lungs are clear and resonant throughout.

Abdomen is pendulous due to excessive fat. No tender points elicited. Spleen and liver are not palpable and apparently are of normal dimensions. There is a slight inguinal hernia on left side. It is easily reduced and causes no pain.

Upper extremities are normal except for two small bluish nodules about 3 mm. wide on the extensor surface of the right hand. Lower extremities reveal a very interesting condition, especially the right leg which has numerous skin tumors varying from 0.5 to 1 cm. in diameter. They are limited to the leg proper and are most numerous about the mesial surface of the ankle and foot. The tumors have a black or bluish color. They are raised about 2 to 3 mm. above the skin surface, feel hard and are slightly painful on pressure. There is one pedunculated tumor between the first two toes which is quite painful. Some of the nodules have disappeared below the right knee leaving only a black or deep brown discoloration of the skin. A few

MULTIPLE HEMORRHAGIC SARCOMA OF KAPOSI*

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The peculiar skin affection known as "multiple hemorrhagic sarcoma" is sufficiently uncommon to warrant the publication of a well studied case, especially when it approaches so closely the description as originally given by Kaposi¹ in 1876. The disease in itself is usually not a fatal one. It occurs after the middle age of life and most often in elderly men. Ordinarily it begins with the appearance of small firm blue nodules on the medial surface of the ankle. This is soon followed by many more nodules which may remain discrete, or coalesce to form a large tumor mass, as occurred in our case. Later, or at the same time, similar lesions may appear on the hands, as in Kaposi's first case, which led him to think that it was a generalized systemic disease. Cases which have come to necropsy have shown nodules also in the lungs, liver, spleen, pancreas, kidney and intestinal tract.

Since Kaposi, many cases have been studied and described in detail. De Amicis¹⁰ published a large series in 1897. Mariani² reported sixty-two cases in 1909 which were reviewed and summarized by Ewing³ in 1922, and several reports of single observations have appeared. Dermatologists have contributed most of the literature on the subject which, however, is of great interest to the general pathologist.

REPORT OF CASE

R. M., a white, male, American, married, confectioner, aged 82 years had, as a chief complaint, painful, ulcerating skin tumors on the medial surface of the right leg.

Past History. Patient has been in good health until his present illness except for "inflammation of right lung" when 22 years of age. He developed a left inguinal hernia eight years ago, which has never caused him any trouble.

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dipping quite deep into the subcutaneous tissue. Directly beneath the rete mucosum, there are fairly well circumscribed groups of spindle cells which can be seen with the naked eye, and vary from 2 to 4 mm. in diameter. The spindle cells are closely packed together and surround many small irregular endothelial-lined spaces, 1 to 2 mm. in diameter, filled with red blood cells. The spindle cells form a stroma for these blood spaces, and have sarcomatous tendencies which are evidenced by the many mitotic figures, the invasiveness and the irregular arrangement. They produce fibroglia. The cells are grouped in fairly narrow strands and course irregularly through the tissue. These circumscribed cellular areas are numerous. Some lie directly beneath the rete mucosum, which as a consequence is abnormally thin, and would possibly ulcerate if growth were not interrupted; while others are found fairly deep in the subcutaneous tissue and over these the epidermis is thickened. There is considerable lymphocytic infiltration with many plasma cells about many of the nodules. Between these tumor nodules are strands of collagen fibers often separated by edema. Numerous blood vessels with thickened walls lie between the collagen fibers. Some of the small capillaries have only a pin-point lumen while others appear completely occluded by swollen endothelium. Verhoeff's elastic tissue stain shows practically no elastic membrane in some of the small blood vessels, while in the larger vessels the continuity of this membrane appears to be interrupted. A normal amount of elastic tissue is found in the subcutaneous tissue beneath the corium, except where the tumor nodules lie directly beneath it, and here there appears to be no elastic tissue. A fairly large amount is found in the connective tissue surrounding the nodules. No elastic tissue appears within the nodules or in the walls of the blood spaces in and around the nodules. The blood vessels show no invasion of their walls by the tumor and, although in places definitely proliferating, the endothelium does not appear to be neoplastic. Practically all the blood vessels have thickened walls but none of them shows thrombosis. There are many areas of hemorrhage in the tissue surrounding the tumor nodules. The hemorrhage is not extensive and is usually found in areas not greater than 5 mm. in width. There is no recent hemorrhage within the nodules. Several nodules consist of very wide closely apposed thin-walled blood spaces just beneath the epidermis, which may readily be designated cavernous hemangiomas. Many

nodules have appeared above the knee during the past month. None of them at present is undergoing ulceration. Some are discrete while, in some areas, many nodules have coalesced to form a large single mass. There is a white scar 15 by 1.5 cm. just below the knee on the mesial surface of the leg where the growth was removed in 1923. Above the scar a few nodules have appeared within the last few months. These have a reddish purple color in comparison with the older lesions on the lower part of the leg. The left leg shows a few tumor nodules irregularly scattered over the lateral surface of the ankle. There is also a large irregular bluish discoloration of the skin about the ankle joint and the lateral surface of the foot. This has appeared within the last year and is not painful or uncomfortable in any way. The right leg appears slightly swollen but there is no marked pitting of the skin after pressure. Pulsation of the dorsalis pedis artery is easily elicited in each foot.

PATHOLOGIC REPORT

Several pieces of tissue have been removed from the leg at different times. The largest one was excised Jan. 25, 1925. It consists of several small nodules which together measure 5 by 3 by 1 cm. (Fig. 1). They are quite firm and have smooth shining bluish surfaces. Individually they vary from 0.3 cm. to 0.6 cm. in diameter and are raised about 3 to 4 mm. above the level of the skin. There are about fifteen such nodules, discrete and with sessile bases. Cross-section of the mass (Fig. 2) shows a dark blue surface consisting of several loculi or blood spaces supported by rather heavy strands of fibrous tissue. The dark areas are limited to the upper portion of the subcutaneous tissue but have a tendency to spread beyond. The remainder of the subcutaneous tissue appears normal. Several other smaller tumors have the same structure as that described above.

MICROSCOPIC REPORT

Sections were fixed in Zenker's fluid and stained with hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, Verhoeff's elastic tissue stain and Foot's reticulum stain. The superficial layers of the skin are very thin, and absent in some places. The rete mucosum appears normal in some parts, while in others there is a marked thickening, with many of the rete pegs

interstitial connective tissue and endothelium which gradually obliterates the blood spaces, forming solid tumors.

It is a question with most of the above writers whether this skin tumor is a true sarcoma. Clinically our case has not the appearance of a rapidly growing sarcoma since it has been present for more than three years and apparently has not injured the health of the patient. This is seldom if ever the case in true sarcoma, which is usually fatal in much less time. The structure of the tumor microscopically, however, leads most pathologists to call it a sarcoma. A fact in this case which favors the diagnosis of sarcoma is that the tumor has spread from the ankle to the region above the knee, and growths are appearing on the other leg. New tumor nodules are slowly developing all the time. A few nodules on the right leg and foot where radium had been applied have retrogressed and only a brownish discoloration of the skin indicates their former site.

The strongest evidence in favor of its essentially malignant nature is the histologic picture. Many unencapsulated foci of spindle cells of fibroblast morphology, with numerous mitotic figures and with definite although not marked invasive qualities, are details which are important in establishing a diagnosis of malignancy. In places the grouping of widely dilated and thin-walled blood sinuses beneath the epidermis is very suggestive of a cavernous hemangioma. From this stage there are gradations through the development of a cellular spindle cell stroma about these vessels, to a preponderance of the spindle cells in the picture. Hence, the possibility must be considered that this is an instance of a sarcoma developing in the stroma of an angioma. This conception finds analogies in certain tumors of the kidney, bladder, uterus and possibly the thyroid gland. However, the subject of mixed tumors which are malignant is a delicate one and dogmatic conclusions should be avoided wherever the origin of the neoplastic cells is in the least doubtful.

In our laboratory we have seen an undoubted instance of a fibrosarcoma arising in the stroma of a carcinoma of the urinary bladder, a case which Foot⁹ included in his study of the reticulum of tumors. Also in the thyroid gland and endometrium we have encountered what we regard as true stromal sarcoma in an adenocarcinoma. It brings up many interesting questions, the old and new problem of a *contagium vivum*, a virus first attacking one type of tissue and then its stroma, or *vice versa*, if indeed it is possible to be certain that both

minute deposits of hemosiderin are present in the connective tissue beneath the corium and surrounding the tumor nodules. The overlying epidermis is atrophic. Several sections stained with Foot's reticulum stain show a fine reticulum developing in the nodules, apparently being laid down by the spindle cells. Nothing suggestive of a fungus or other infectious agent is seen in any of the sections.

DISCUSSION

This case from a clinical standpoint corresponds very closely with those originally described by Kaposi. Since then many others have been reported. Only ten cases with complete necropsies have been reported in this country. All of them showed thoracic and abdominal metastases, and Philippson⁴ found lesions on the conjunctivae, tonsils, and soft palate. X-ray plates of the thorax of our case show no evidence of lung involvement.

Various interpretations have been given of the histopathology of this condition. Some writers believe it is purely a sarcoma while others consider that it was originally a granuloma which later became malignant. Dillard and Weidman⁵ found a fungus with structures identical with those of *Achorion schoenleinii*. They do not believe this to be the cause of the disease, but think it should be considered and searched for in all cases which come to necropsy. They believe that there is some general systemic influence which induces a dilatation of the lymph and blood vessels in the peripheral parts, with inflammatory agents entering later to produce the hyperplastic features of the stroma. Ewing believes it to be an infectious granuloma of unknown origin, which in its later stages acquires genuine neoplastic properties. Dillard and Weidman endeavored to produce similar lesions in rabbits by feeding them cultures of *Achorion schoenleinii* and injecting cultures intraperitoneally with no positive results. On the other hand Justus⁶ claims to have inoculated white mice with tissue from a case of Kaposi's sarcoma, with the production of systemic lesions which were carried through five generations of white mice. Frost⁷ reports one case and tends to the belief that the lesions have an inflammatory basis. Gilchrist and Ketron's⁸ studies lead them to consider that it is due to a proliferation and dilatation of the blood capillaries which are very frail and liable to rupture, thus stimulating a proliferation of

PLATE 95

FIG. 4. Mesial surface of the right leg showing old and recent lesions, and the scar where the tissue in Fig. 1 has been removed, also several recently developed nodules near the middle of the thigh.

FIG. 5. A portion of one lesion, the typical picture of a cavernous hemangioma. Hemosiderin granules in the subcutaneous connective tissue. Papillae of corium absent. Thickening of the horny layer of epidermis with desquamation. $\times 250$.

PLATE 96

FIG. 6. A lesion, fairly well circumscribed, showing an abundance of dilated blood spaces, with beginning spindle cell reaction in the stroma. The endothelium in many of these vessels is swollen and proliferating. Lymphoid, plasma cells and large mononuclear cells are in great number. $\times 80$.

FIG. 7. A lesion more advanced toward a purely spindle cell tumor than that in Fig. 6. Marked edema of the surrounding stroma.

PLATE 97

FIG. 8. A low power view of a spindle cell tumor close to the skin surface, causing atrophic changes in the latter. Many thin-walled vessels about the periphery of the nodule.

FIG. 9. High power photomicrograph of a field in Fig. 7, stained with Mallory's phosphotungstic acid hematoxylin, showing the spindle cells with well defined fibroglia fibrils. Three mitotic figures are visible.

tissues were not affected simultaneously. Burrows' hypotheses, involving primarily local nutritional changes in the genesis of neoplasms, offer a logical method of approach to the explanation of malignant changes in the stroma of epithelial and other primarily non-fibroblastic tumors.

SUMMARY

1. A case is reported of "multiple hemorrhagic sarcoma of Kaposi," in a man aged 82 years, who has had the condition at least three years. The history and clinical findings are in agreement with most of the recorded cases.

2. The disease is rarely fatal, hence its clinical course is in striking contrast with most cases of sarcoma.

3. Careful histologic study of the lesions indicates that the condition is probably one of multiple hemangiomatosis with a fibrosarcomatous change in the stroma of many of the tumor nodules.

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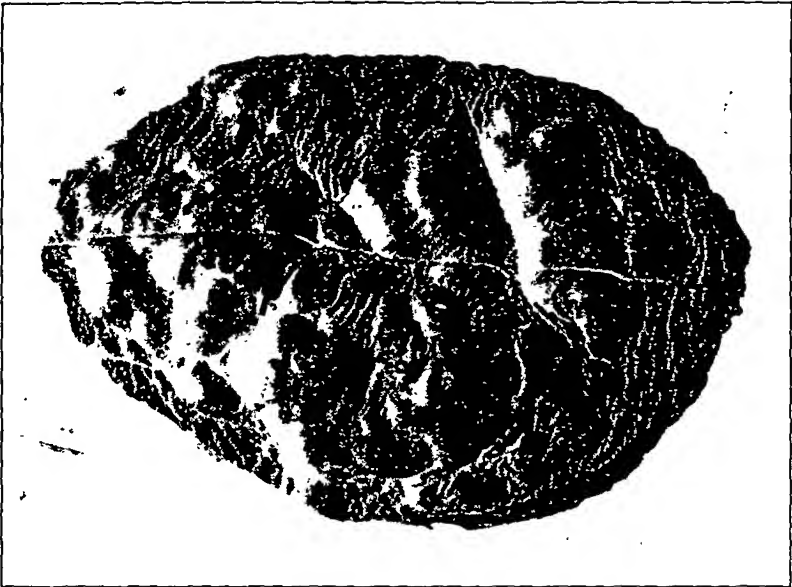
DESCRIPTION OF PLATES

PLATE 94

FIG. 1. Conglomerate tumor nodules, actual size, removed from mesial surface of the right knee, Jan. 25, 1923.

FIG. 2. Cross-section of tumor mass in Fig. 1, slightly enlarged, showing the cavernous hemorrhagic appearance of many of the nodules, some of which are beginning to invade the underlying fat.

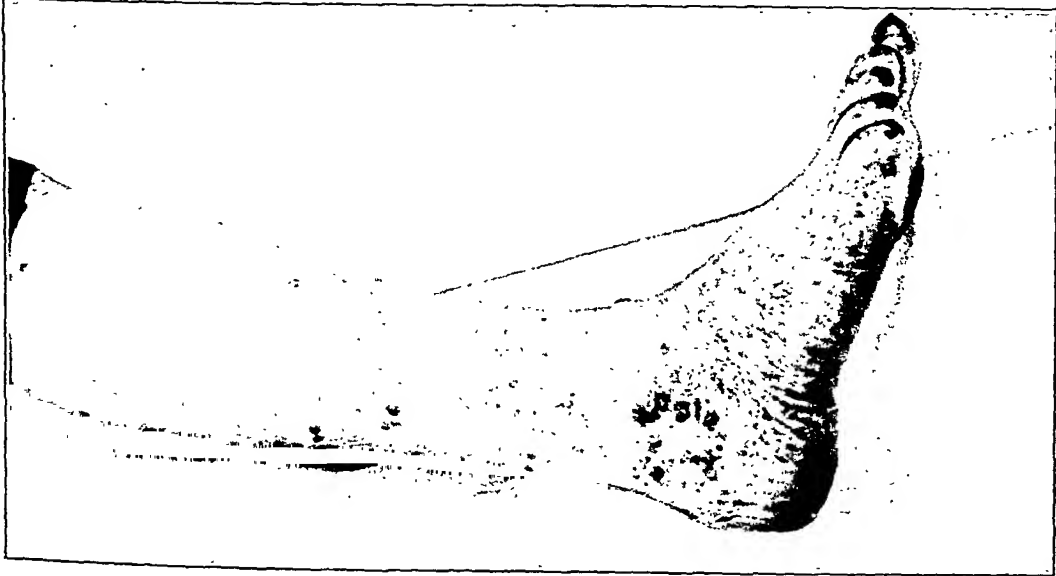
FIG. 3. Lateral surface of the right leg and foot, Dec. 18, 1926, showing the distribution of the lesions, and cyanosis of the foot.



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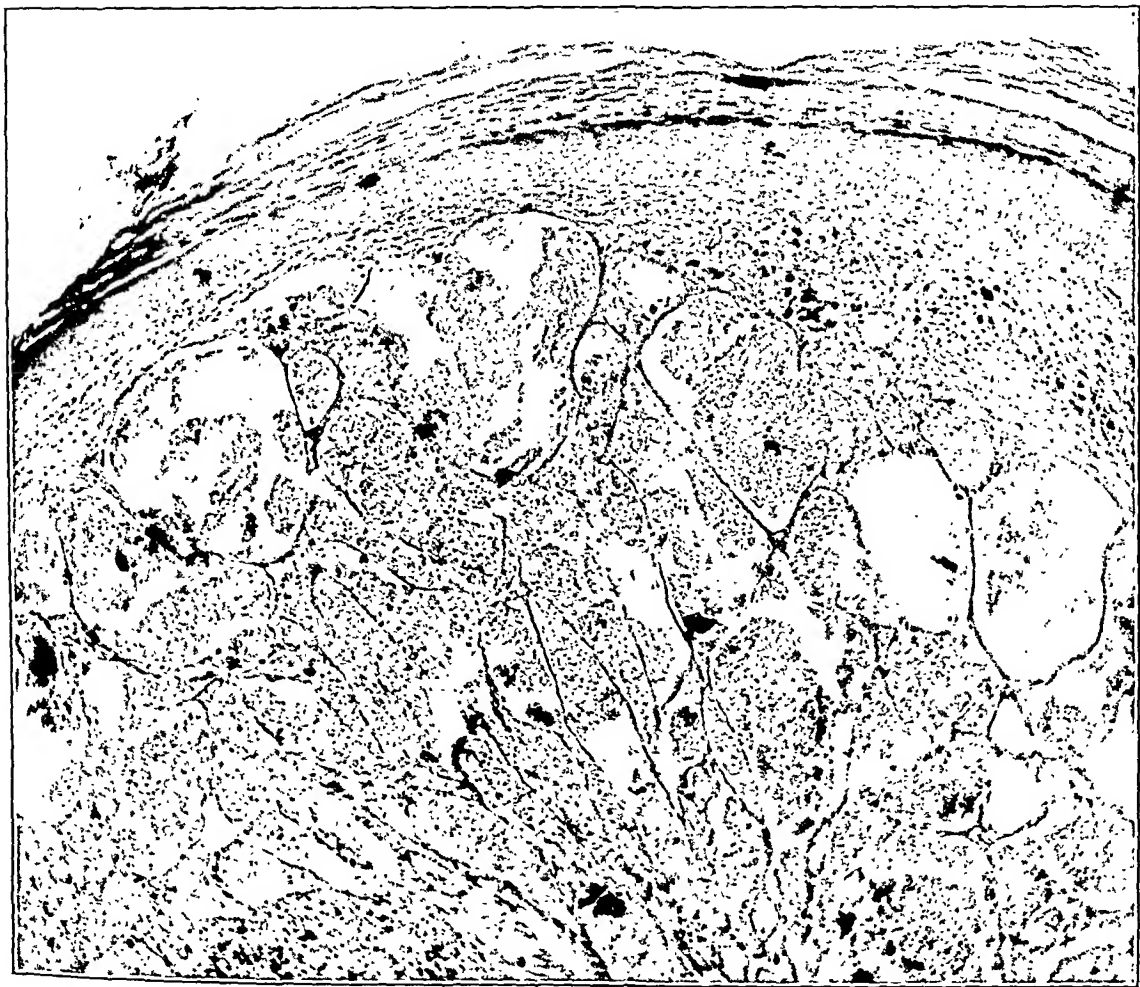
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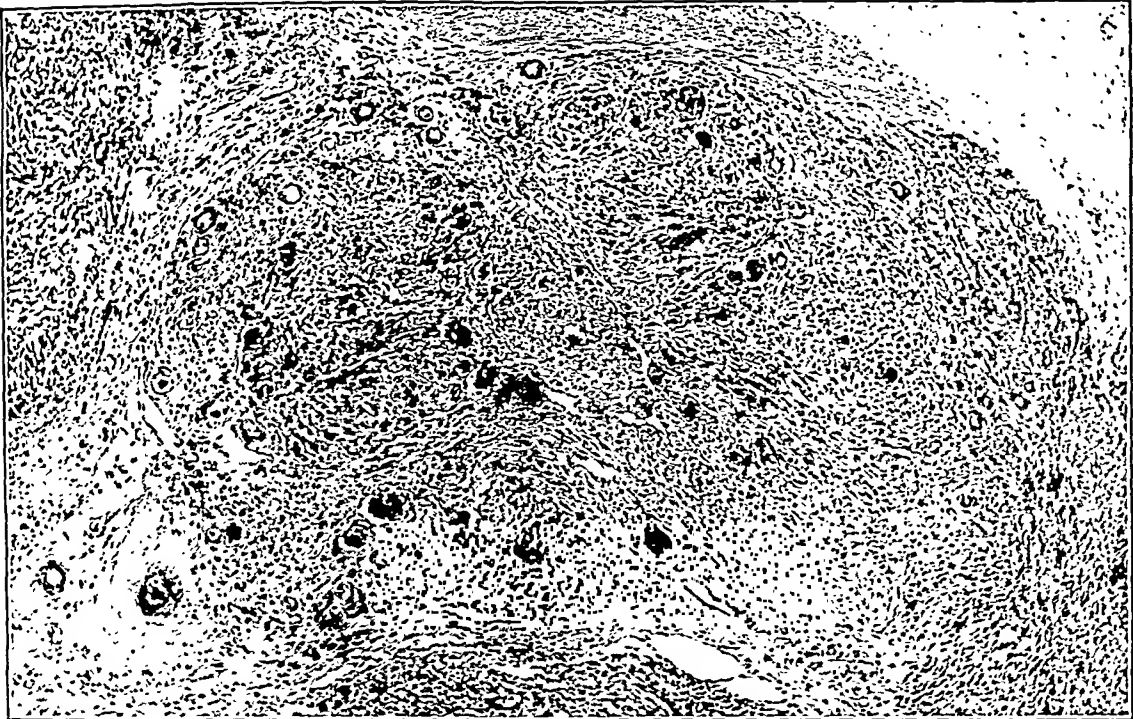
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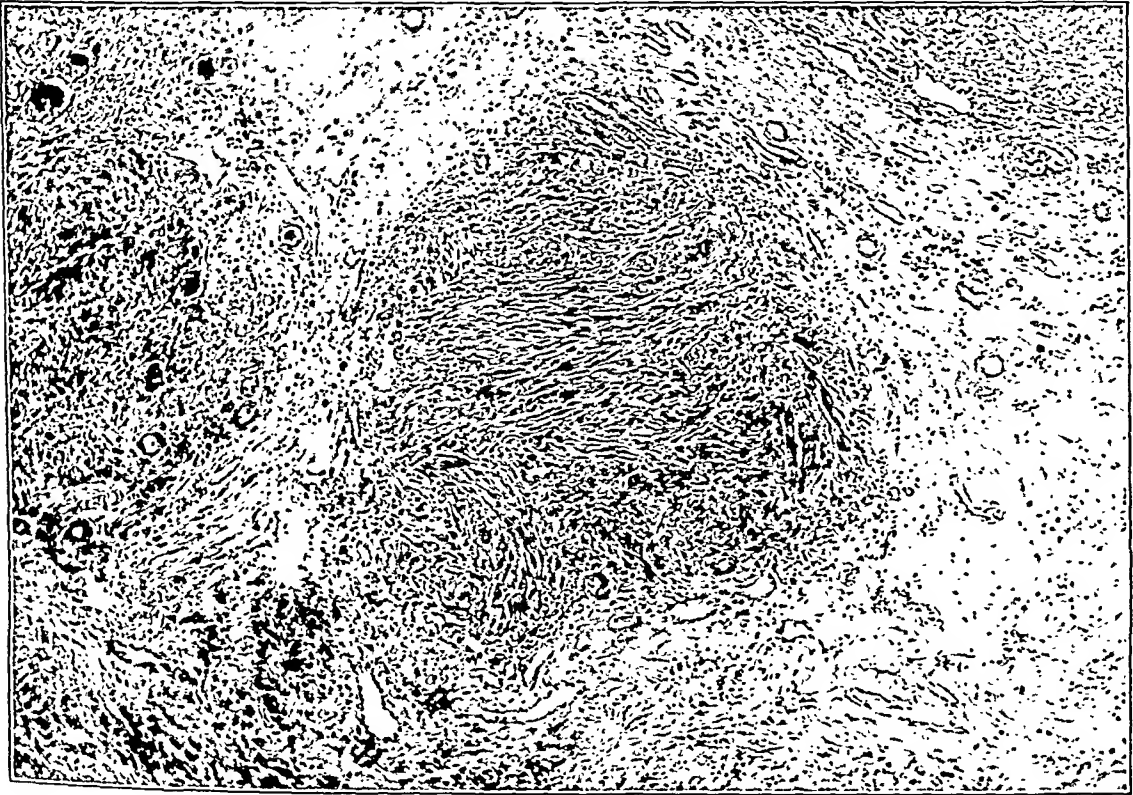
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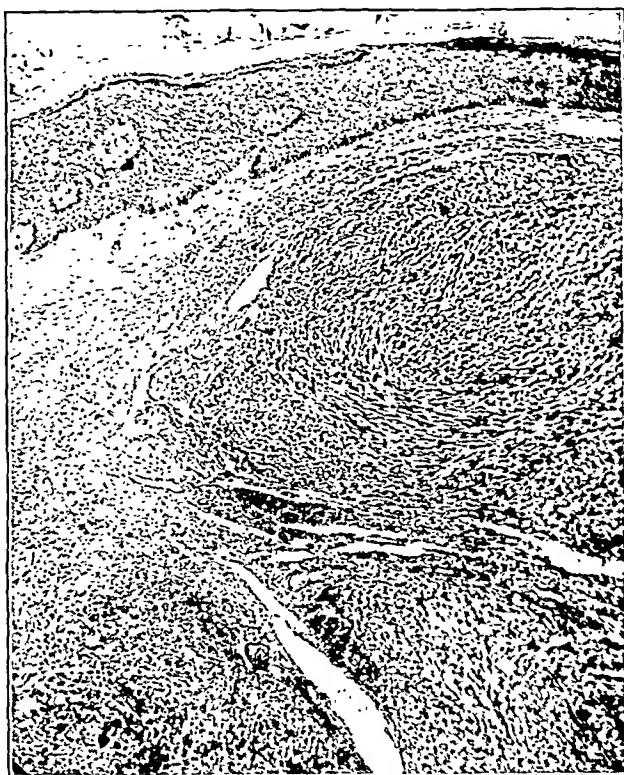
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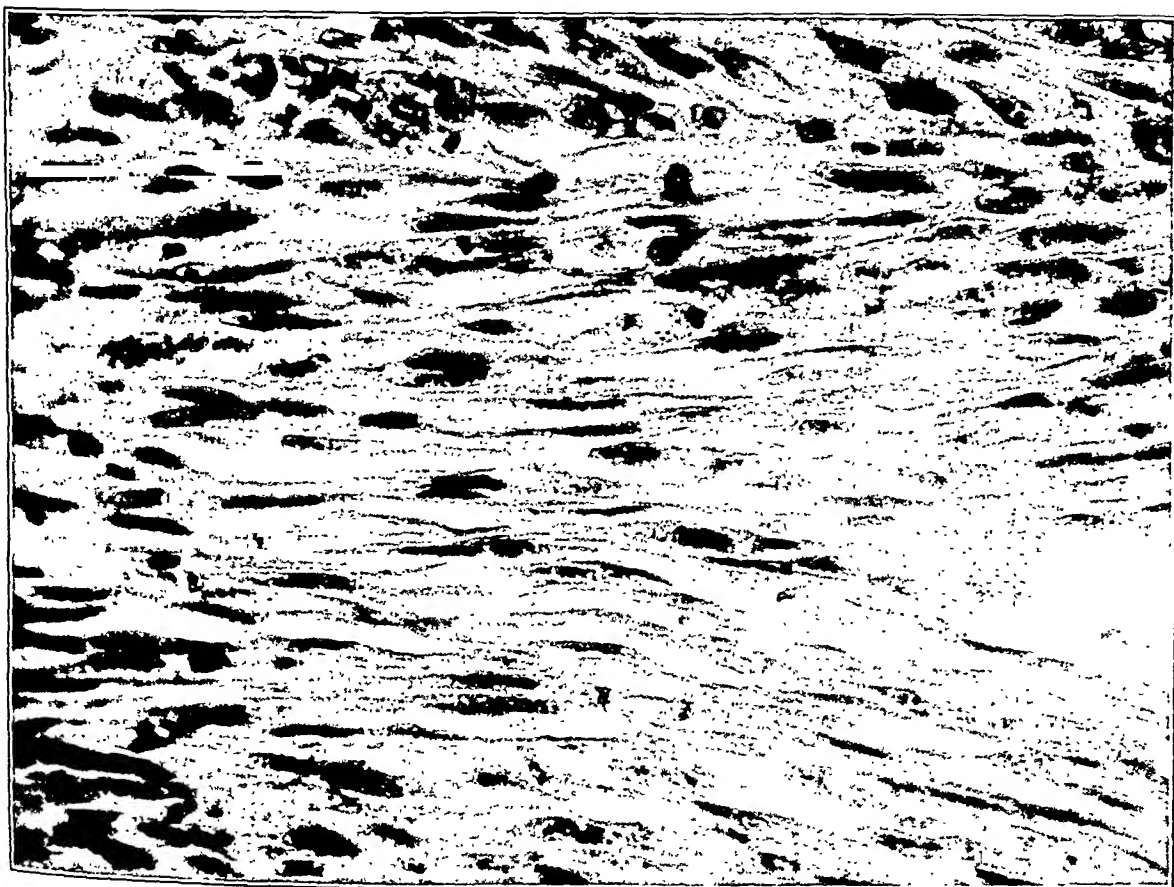
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Rabbit C three weeks; Rabbit D six weeks; Rabbit E twelve weeks; Rabbit F eighteen weeks; Rabbit G twenty-eight weeks and Rabbit H twelve months. The left kidney removed at necropsy was fixed in 10 per cent formalin and embedded in paraffin. Serial sections were made of all these kidneys, except those of Rabbits A and H; and parts of these were used for histologic examination for fat and connective tissue. The serial sections were cut 6 microns thick, perpendicular to the long axis of the kidney, and every tenth section was taken. The sections were stained with hematoxylin and eosin; for fat, Sudan III was used; and for connective tissue, Mallory's anilin blue. All the glomeruli in every section were counted. Two glomeruli from the periphery, and two from the central portion of the cortex of each section were measured. Only glomeruli showing a hilum were measured, and measurements were made only of the transverse diameter of these glomeruli. A euscope was used to facilitate the counting, and a filar micrometer ocular was used for measuring.

Serial sections of three rabbit kidneys were used as controls. One of them was the normal right kidney (kidney X), removed from a rabbit (Rabbit C), whose second kidney was allowed to become hypertrophic. The second kidney (kidney Y) was the left kidney taken from a normal rabbit of the same weight as Rabbit F, just before the latter was killed. The third kidney (kidney Z) used as control was the hypertrophic left kidney, taken from a rabbit after the ureter of the right side had been ligated. This was done to determine whether or not removal of one kidney produced the same degree of hypertrophy as ligation of a ureter. The ureter of this rabbit was ligated at the same time that one kidney from rabbit F was removed. Both rabbits were killed after eighteen weeks, in order to allow the kidneys the same length of time for hypertrophy.

RESULTS

Table No. I gives the weights of the rabbits before extirpation of one kidney, and at the time of death. It further shows the absolute and relative weights of the normal and hypertrophic kidneys and gives the percentage increase in the relative weight of the hypertrophic kidney over the normal. The control kidney Y weighing 7.93 gm. appears to be just the upper limit for the normal weight of a kidney of a rabbit weighing approximately 2600 gm. Brown² and

THE STATE OF THE GLOMERULUS IN EXPERIMENTAL HYPERTROPHY OF THE KIDNEYS OF RABBITS

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INTRODUCTION

It is a well established fact that the removal or injury of one kidney will, under favorable circumstances result in hypertrophy of the other. Before 1900 many investigators believed that hyperplasia was the underlying factor in the enlargement of the kidney. They described the formation of new glomeruli and the budding of tubules. In 1916, Hinman⁴ in his admirable study of renal counterbalance gives the complete literature of this subject. Galeotti and Villa-Santa³ were the first to investigate carefully hypertrophic kidneys. They arrived at the conclusion, that, following the removal of one kidney in young animals, there is a genuine hyperplasia of the glomeruli and tubules of the remaining kidney. Full-grown animals, however, show under similar conditions only plain hypertrophy. Until the present time, there is no contradiction to these statements, and even Hinman says that since 1900 no one, so far as he knows, has expressed a belief in true renal hyperplasia, except in the case of very young animals.

In a recent investigation performed in collaboration with Karsner and Todd,⁶ it was shown that the enlargement of the heart in hypertrophy is due principally to a hypertrophy of the muscle fibers without hyperplasia. It became of interest then to investigate the state of the glomeruli in hypertrophic kidneys, more especially those of young animals, to see if a true hyperplasia of the glomeruli occurs.

MATERIAL AND METHODS

Eight white rabbits of the same litter were used for this experiment. Under local anesthesia, the right kidney was removed; and after various intervals of time the rabbits were killed. Rabbit A was killed three days after removal of the kidney; Rabbit B eight days;

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series as to the actual increase in weight of kidneys following unilateral nephrectomy, and further research along these lines is suggested.

SIZE OF GLOMERULI

Studying a series of slides of normal and hypertrophic kidneys, it is evident that there is a marked difference in size between the glomeruli in the periphery of the cortex (peripheral glomeruli), and the glomeruli closer to the medulla (central glomeruli). This observation has already been made by Kuelz⁸ in kidneys of new-born infants.

Normal Kidneys. Kidney X weighed 2.62 gm. (weight of rabbit 850 gm.). Kidney Y weighed 7.93 gm. (weight of rabbit 2650 gm.). Table II gives an analysis of the figures and Fig. I shows the figures graphically.

TABLE II

The Sizes of the Peripheral and Central Glomeruli of two Normal Kidneys

Unit of measurements of filar micrometer *	Number of glomeruli			
	Kidney X		Kidney Y	
	Peripheral glomeruli	Central glomeruli	Peripheral glomeruli	Central glomeruli
51-60.....	6
61-70.....	185	44
71-80.....	137	199	88	..
81-90.....	4	81	238	111
91-100.....	..	8	230	294
101-110.....	151

* Measurements are given in units according to the subdivision of the filar micrometer; 10 units are equal to 0.008 mm.

Kidney X shows small and large glomeruli. The central glomeruli are definitely larger in size. Only four peripheral glomeruli measure 81-90 units, while eighty-one central glomeruli measure over 80 units. The peaks of Fig. I (Kidney X) are given by 185 peripheral glomeruli measuring 61-70 units, and 199 central glomeruli measur-

his co-workers give 13.67 gm. as the normal weight of both kidneys in rabbits of 2638 gm. In four normal rabbits weighing approximately 2600 gm. each, we found the weight of the left kidney to be 6.02; 6.63; 6.91 and 7.03 gm. respectively.

TABLE I

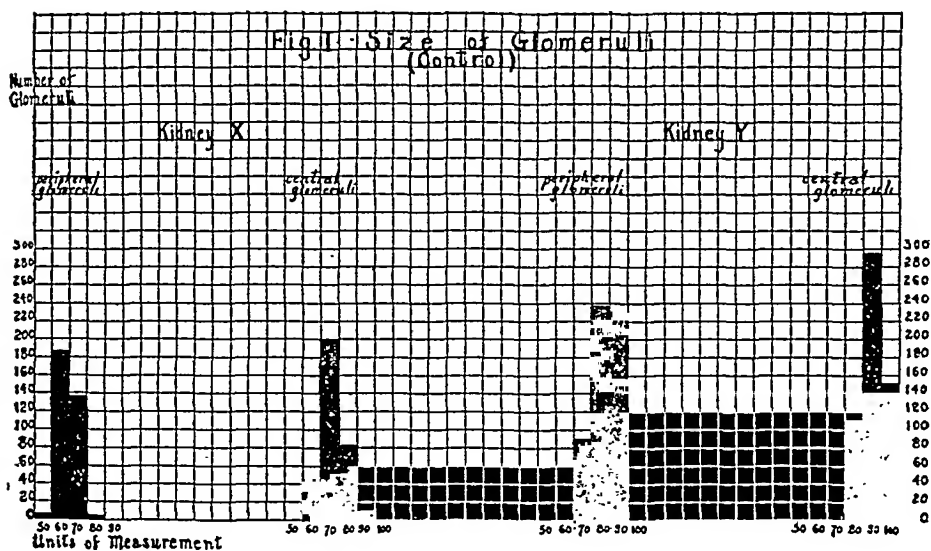
A Comparison between the Weights of the Rabbits before Extirpation of one Kidney, and at the Time of their Deaths; the Absolute and Relative Weights of Normal and Hypertrophied Kidneys and the Percentage in Relative Weight of the Latter over the Normal

Rabbit number Period of hypertrophy	Weight of rabbit		Absolute weight of kidneys		Relative weight of kidneys		Increase in relative weight of hypertrophic kidneys over normal
	Before operation	After death	Normal	Hyper- trophic	Normal	Hyper- trophic	
	gm.	gm.	gm.	gm.	per cent	per cent	per cent
A 3 days.....	410	410	2.26	2.41	.551	.588	6.7
B 8 days.....	520	540	2.34	2.57	.450	.476	5.8
C 3 weeks.....	850	900	2.62	2.9	.308	.322	4.5
D 6 weeks	975	1235	2.59	4.43	.268	.358	33.6
E 12 weeks....	925	1350	2.91	5.32	.314	.394	25.5
F 18 weeks....	1000	2650	3.46	9.01	.346	.340	-1.73
G 28 weeks....	1050	2825	3.28	9.28	.312	.328	5.1
H 12 months..	950	3300	2.80	9.35	.295	.283	-4.1
X (Control)...	850	..	2.62	..	.308
Y (Control)...	2650	..	7.93	..	.300
Z (Control) 18 weeks....	975	2574	..	8.96	..	.348	..

According to Table I, it seems that for twelve weeks after unilateral nephrectomy, the remaining kidney shows an absolute and relative increase in weight. After that interval, the relative weight of the kidney practically remains stationary. There are, however, so many variable factors, such as the different weights of the kidneys before operation, the different weight and size of the rabbits, that it is by no means permissible to draw any conclusion from this small

ing 71-80 units. Kidney Y shows similarly large and small glomeruli; 230 peripheral glomeruli measure 91-100 units and no peripheral glomeruli are found which measure 100-110 units; 294 central glomeruli measure 91-100 units and 151 central glomeruli measure 101-110 units.

Hypertrophic Kidneys. Table III is the analysis of the figures and Figs. II and III represent them graphically. The more hypertrophic the kidneys, the larger are the glomeruli. The peaks of the figures representing the peripheral glomeruli move from 70 units



to 80 and to 90, but then stay there. The majority of the peripheral glomeruli of kidneys of twelve and twenty-eight weeks hypertrophy have a diameter of 90 units. Of the remainder, in the more hypertrophic of the kidneys, most of the peripheral glomeruli are larger than 90 units; and in the less hypertrophic of the kidneys they are smaller. The peripheral glomeruli of the hypertrophic kidneys are much more uniform in size than those of the normal kidneys.

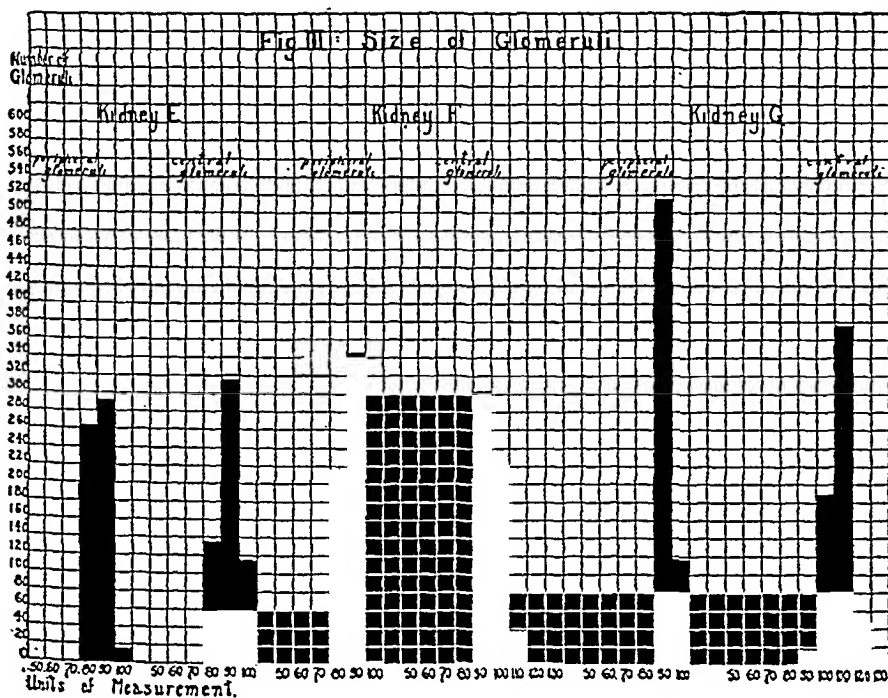
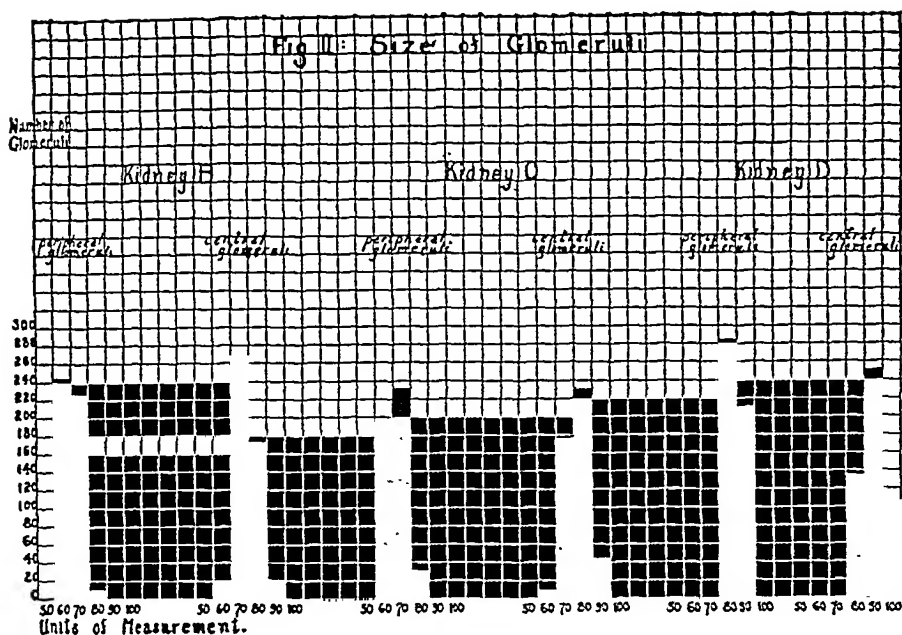
The peaks of the figures showing the sizes of the central glomeruli, vary from 80 to 110 units, according to the degree of hypertrophy. In the hypertrophy of twenty-eight weeks, there is less uniformity in the size of the central glomeruli than of the peripheral glomeruli. Such a uniformity might develop after a longer period. The figures show further that the more hypertrophic the kidneys the greater is

the difference between the sizes of the peripheral and central glomeruli. While, for instance, in the normal kidney the difference between the larger peripheral glomeruli and central glomeruli does not exceed 10 units, the difference in hypertrophic kidneys may reach 30 units. Or, in other words, some of the central glomeruli in hypertrophic kidneys are about one-fourth larger than peripheral glomeruli.

The difference in size between the peripheral and central glomeruli has already been demonstrated in normal kidneys. It is known that the arteries enter the cortex from the medulla and extend from there toward the periphery of the cortex (interlobular arteries). These arteries give off at intervals the afferent vessels of the glomeruli, so that the glomeruli near the medulla are supplied first and those at the periphery later. We assume that the central glomeruli, receiving the blood before the peripheral glomeruli, get a larger amount of filterable material, and this, because of increased work, is sufficient to produce a slight hypertrophy of the central glomeruli. Morison,⁹ who believes that some of the glomeruli near the medulla are larger, states that these glomeruli are supplied by the arcuate arteries. In hypertrophic kidneys both the central and the peripheral glomeruli are enlarged. Assuming that both types of glomeruli were increased in the same ratio, the difference in the size of the peripheral and central glomeruli naturally would be greater the more hypertrophic the kidneys.

It is known that in kidneys showing chronic glomerulonephritis a few glomeruli always appear normal and a few hypertrophic (Karsner⁵), a fact that is not explained. The hypothesis is offered that these glomeruli are the ones near the medulla. These being somewhat larger normally may show a greater resistance to pathologic changes and therefore appear unaffected and hypertrophic.

Comparing the size of the glomeruli of the control kidney Y and kidney F (both rabbits have the same weight), it is clear that the glomeruli of the hypertrophic kidney F are definitely larger. Of the peripheral glomeruli of kidney F, 344 measure 91-100 units, while only 230 peripheral glomeruli of Rabbit Y measure 91-100 units. Of the central glomeruli of kidney F, 221 measure 101-110 units as compared with 151 central glomeruli of the normal kidney. Control kidney Z (with ligation of ureter) shows essentially the same sizes of glomeruli as kidney F.



weights of the rabbits and kidneys vary greatly. Kidney Y apparently shows more glomeruli, but that point will be taken up subsequently.

Hypertrophic Kidneys. Fig. V and Table V analyze the numbers of the glomeruli counted in hypertrophic kidneys and the chart shows the graphic figures of these numbers.

TABLE V

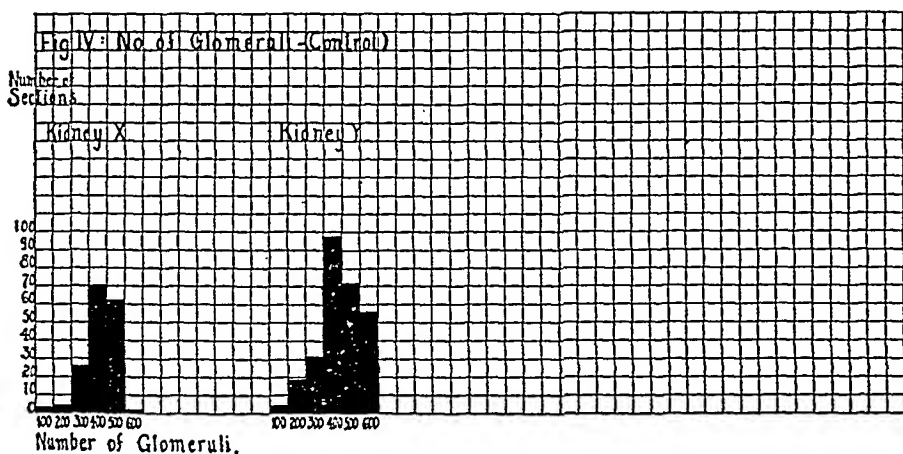
The Number of Glomeruli Actually Counted in Sections from six Hypertrophied Kidneys

Number of glomeruli	Number of sections					
	Kidney B	Kidney C	Kidney D	Kidney E	Kidney F	Kidney G
1-110.....	..	5	2	1	2	5
101-200.....	11	10	10	3	3	8
201-300.....	11	6	16	8	16	9
301-400.....	34	30	20	31	21	19
401-500.....	97	38	34	16	32	45
501-600.....	74	94	102	89	56	67
601-700.....	13	40	62	127	131	148
701-800.....	..	5	..	8	21	16

As in the case of the control rabbits, the contours of the graphic pictures, indicating the number of the glomeruli, are essentially the same in the various hypertrophic kidneys. There seem to be more glomeruli present the more hypertrophic the kidneys, as shown by the various peaks of the pictures. However, this is not due to an actual increase in the number of glomeruli, but to the fact that the glomeruli are much larger and some of them, therefore, are present and counted in two succeeding sections. The majority of the glomeruli of the smaller control kidney X show a diameter of 60 and 70 units, equal to 0.048 mm. and 0.056 mm. The sections are 0.006 mm. thick, and every tenth section was taken. The space between two sections therefore, measures 0.06 mm., indicating that the majority of the glomeruli were counted only once in this particular

NUMBER OF GLOMERULI

The numbers of glomeruli represent the actual number counted. These numbers, of course, do not indicate the absolute number of



glomeruli, but merely give a relative figure which may be used only for comparison with the number of glomeruli of the different kidneys.

Normal Kidneys. Table IV analyzes the number of glomeruli in the two normal kidneys and Fig. IV gives the graphic picture.

TABLE IV

The Number of Glomeruli Actually Counted in Sections from the two Normal Kidneys

Number of Glomeruli *	Number of sections	
	Kidney X	Kidney Y
101-200.....	3	4
201-300.....	4	18
301-400.....	26	31
401-500.....	69	97
501-600.....	62	72
601-700.....	2	56

* Sections containing less than 100 glomeruli are not registered in this chart.

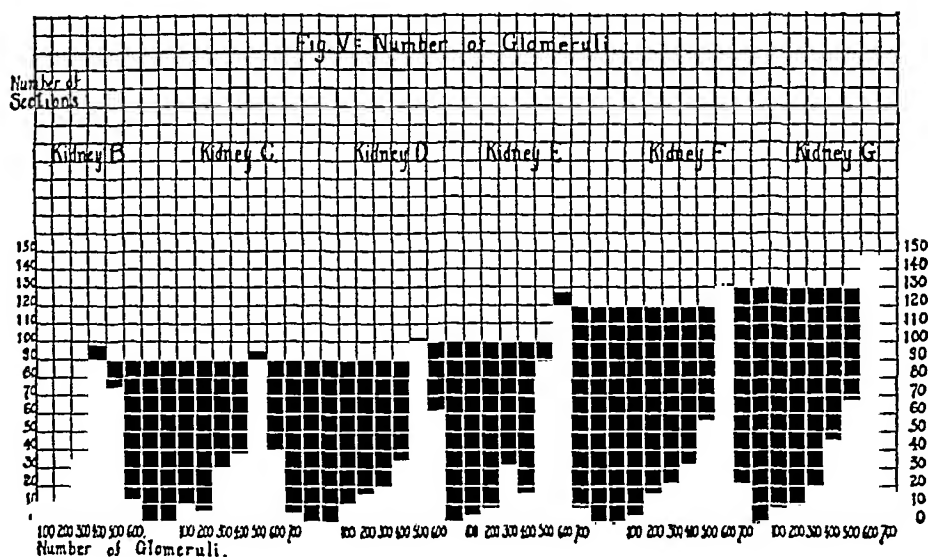
Looking at the chart it appears that both curves indicating the numbers of the glomeruli are essentially the same, even though the

similar to those of kidney A. The vessels throughout are dilated and filled with red blood corpuscles. There is diapedesis of red blood corpuscles around the vessels.

The hypertrophic kidney of three weeks' duration shows no distension of the glomeruli, and the loops show nothing unusual. The capsular space, however, contains an abundant amount of a serum precipitate which in some places fills out the entire subcapsular space. It is of interest to observe that most of the serum precipitate in the capsular space is found just opposite the hilum. The precipitate appears triangular, with its apex pointing toward the hilum of the glomerulus. The form of the serum precipitate is very characteristic and is demonstrated in every section. These changes are found uniformly throughout the sections. The lumen of the convoluted tubules contain a similar serum precipitate. The lining cells still show cloudy swelling of their cytoplasm, to a much less extent, however, than the sections of the previous kidneys. There is no noticeable hyperemia.

Sections of the kidney of six weeks' hypertrophy and the sections of the remainder of the series show practically no changes, and apart from the increase in size of the glomeruli, it would be impossible from the histologic appearance to state that the sections were taken from hypertrophic kidneys. Occasionally a slight amount of serum precipitate is found in the capsular space of the glomeruli in the vicinity of the medulla. Only a few dilated and hyperemic blood vessels can be made out. None of the sections shows mitotic figures, and a very careful search shows no definite signs indicating a multiplication of either glomeruli or tubules. Sections of kidney A and kidney H (three days' and twelve months' hypertrophy respectively) contain no fat and show no increase in connective tissue. It may be especially emphasized that hypertrophic kidneys of twelve months' duration do not show fat either in the glomeruli or in the convoluted tubules. Textbooks (Kaufman,⁷ Aschoff¹) repeatedly state that the fat content of apparently normal or hypertrophic tubules and glomeruli in kidneys showing nephrosclerosis of the arteriolar variety, is due to the overwork of these areas. The fact that hypertrophic kidneys of twelve months' duration show no fat at all seems to indicate that the fat content of such areas is not due to overwork alone.

kidney. In hypertrophic kidneys, the glomeruli become much larger and some glomeruli must have been counted twice. We think that the essential similarity of the pictures alone indicates that there is



no absolute increase in the number of glomeruli. The control kidney Z (with ligation of ureter) shows essentially the same number of glomeruli as kidney F.

HISTOLOGIC CHANGES OF THE GLOMERULI

In sections of the left kidney of Rabbit A (three days' hypertrophy), the capillaries of the glomeruli are distended with red blood corpuscles. The glomeruli fill out almost the entire capsular space which shows in a few places only, a slight amount of an acidophilic granular material. The lining cells of the convoluted tubules show marked cloudy swelling. No mitoses are seen, which is contrary to the observations of Hinman. The blood vessels throughout are hyperemic, but no signs of diapedesis of red blood corpuscles are seen.

Eight days after nephrectomy, the glomeruli of the remaining kidney are still distended with dilated capillaries and, in addition, contain a small amount of amorphous pigment in the loops of the tufts. A few red blood corpuscles are present in the capsular spaces. The glomeruli nearer the medulla show these changes more markedly than those nearer the cortex. The convoluted tubules show changes

DESCRIPTION OF PLATE

PLATE 98

- FIG 1. The characteristic shape of the serum precipitate in the capsular space of the glomeruli in kidneys three weeks after unilateral nephrectomy.
- FIG 2. Peripheral glomeruli in kidneys eighteen weeks after unilateral nephrectomy.
- FIG. 3. Central glomeruli in kidneys eighteen weeks after unilateral nephrectomy.

SUMMARY AND CONCLUSIONS

The glomeruli of kidneys of normal rabbits are larger in the vicinity of the medulla than in the peripheral portion of the cortex. In hypertrophic kidneys the majority of the glomeruli are enlarged and the differences in size between the glomeruli of the peripheral and central portions of the cortex appear greater. No signs of new formation of glomeruli or tubules in young rabbits are seen. The enlargement of kidneys of rabbits in experimental hypertrophy is due in part to a hypertrophy of the glomeruli without an increase in the number of the glomeruli. No attempt was made to measure the tubules in hypertrophic kidneys. The hypertrophy of a kidney which followed the ligation of the ureter of the other kidney was no greater than that of the unilaterally nephrectomized animals. During the first three weeks of hypertrophy, the kidneys show, first, a hyperemia of the capillaries and, later on, a marked cloudy swelling which probably reaches its maximum about three weeks after unilateral kidney extirpation. Later on, the changes disappear and the hypertrophic kidneys show no degenerative changes. Hypertrophic kidneys show no traces of fat.

I am deeply indebted to Dr. Howard T. Karsner for the accompanying photomicrographs and also for his valuable suggestions.

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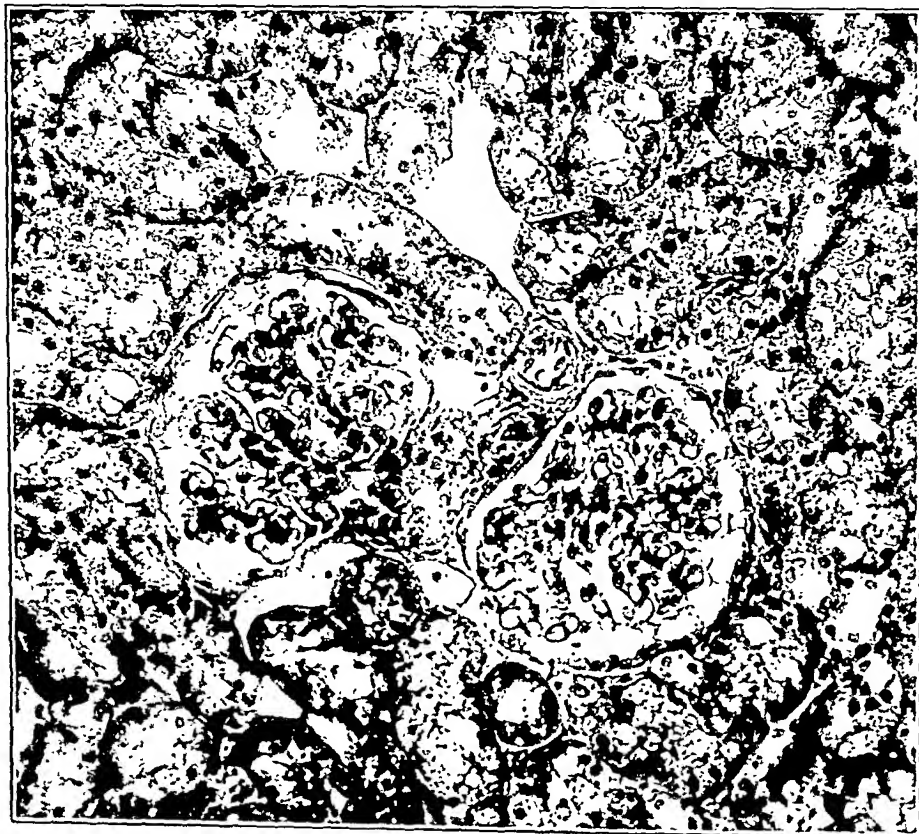
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1



2



3

Saphir

Experimental Hypertrophy of Glomerulus

reasons which will be brought out in the discussion of this paper, the writer will hereafter refer to the groups of cells under consideration as the *sympathicotropic cells*.

Bucura ⁴ in 1907, appears to have been the first to describe these cells and to illustrate them. He regarded them as chromaffin cells, primarily on a morphologic basis. He found the cells in the ovarian hilum of a woman 55 years old, whose ovaries had been removed as a therapeutic measure for osteomalacia. The cells were associated with the large nerves and vessels. He described unipolar ganglion cells among the large cells which he called "chromaffin cells."

Berger's elaborate paper was published in 1923 and deals mainly with the cells as found in adults, to which the name "glande sympathicotrope" was applied. He reported numerous instances, including several from pregnant women. He found these cells in the ovaries of one woman 80 years of age. It seems that his terminology is based upon the close relationship of these large cells with the sympathetic nerves. Berger found his "glande sympathicotrope" not always within or near the nerve sheaths, but at times somewhat removed therefrom, and occasionally lying in the ovarian stroma, yet near the hilum. By special technic, he found the chromaffin, argentaffin and sideraffin reactions invariably negative, but fat or lipid globules were generally present. He described and illustrated coarse granules, pigments and crystals in the cytoplasm. Berger did not regard the cells as chromaffin foci as Bucura and de Winiwarter,^{10, 11} had, and even questioned the occurrence of the ganglion cells which Bucura described.

Schäfer ⁸ in his text merely mentions these cells as occurring in the mesovarium, and names them "interstitial cells" recalling the interstitial cells of the testis. Lewin,⁶ who has recently reported the same cells in the ovaries of two elderly women, calls them "interstitial gland cells" apparently after Schäfer. Lewin found lipid pigments in the cytoplasm and intimated a rather fantastic rôle for the cells.

Turning to the works of others, especially those of Aschoff,¹ de Winiwarter and Kohn,⁵ one finds strong evidence for the chromaffin nature of these nests of cells occurring in the ovarian and testicular hila. In 1903, both Aschoff and Kohn published long papers describing chromaffin cells in the testes and ovaries of fetuses and in the new-born. de Winiwarter's work in 1910 confirmed that of

THE SYMPATHICOTROPIC CELLS OF THE OVARY AND TESTIS*

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During the course of the routine microscopic examination of ovaries removed at operation, certain groups of large cells morphologically resembling chromaffin cells, associated with the non-medulated nerves, were observed in the hilum of an ovary. Stimulated by the finding of a supposedly unusual structure in this site, careful examination was made of all the ovaries sent to the laboratory during the following two years, and approximately twenty-five examples of these cells occurring in one or both ovaries were found. About two hundred specimens of ovaries, singly and in pairs, were studied. Most of the material came from young women with pelvic inflammatory diseases. Several of the ovaries were essentially normal. Some were obtained at necropsy. The material came from individuals ranging in age from a few weeks to sixty years. To insure a constant method of examination of the ovary, sections were cut at right angles to the long axis, and as much of the mesovarium included as was free from trauma. The tissues were fixed in Zenker's fluid and stained with eosin and methylene blue.

Later, the region of the hilum of the testis was examined in a number of instances, and in six specimens similar cells were found. All the testicular material came from adults, and with one exception was obtained at necropsy.

LITERATURE

Few papers dealing with these cells are found in the literature. They have been variously named "chromaffin cells," "cellules pheochromes," "glande sympathicotrope," "interstitial gland cells," etc. No extensive studies of these cells have been published in English, although Lewin⁶ reported his observations in two individuals. By far the most comprehensive paper concerning these cells in adults was written by Berger² in 1923. Other articles may have been written and not found on account of the varied terminology. For

* Received for publication April 5, 1927.

Frequently, one finds only a few cells in a given ovary and these perhaps are within a nerve sheath. In another instance, sufficient cells are seen within the nerve to cause a separation of its fibers and a fusiform enlargement of the nerve. In the same ovary, or even in the same section, the cells may be found surrounding the nerve very much as a mantle of wandering cells. Still other sections reveal groups of cells just touching or near a nerve, while occasionally large groups of these cells are quite removed from the nerves. In some specimens the cells are scattered along the course of the nerve, and at times sprinkled loosely among the nerve fibers. It is difficult to demonstrate any close relationship between the nerves and the group of cells occasionally seen in the ovarian tissue proper. One observes no genetic relationship with the nerves or their sheaths, other than their close association with the nerves.

The sympathicotropic cell groups show a more than casual association with the lymphatics and blood vessels. In many instances the cell masses lie along the lymphatics and are separated from them only by endothelium. The lymphatic vessels about the larger cell clusters are frequently abundant and the larger clusters of cells are well supplied with small blood vessels.

There does not appear to be a close association between the cell masses and the rudimentary tubules of the ovarian hila, as described by others, particularly in embryologic and fetal material. The cells are never found in the broad ligaments, mesosalpinx or parametrium, yet chromaffin cells have been frequently observed in the broad ligaments of fetuses by others.

The groups and clusters of cells, whether inside or outside the nerves, are generally closely packed. Those outside the nerves lie freely in the loose connective tissue of the mesovarium. A limiting membrane about the masses of cells is absent. At times the more peripheral cells of a cluster are somewhat separated from the main mass, but this is not a common feature. Very often the large groups of cells are just under the serosa covering the mesovarium.

The sympathicotropic cells are large polyhedral, round or oval cells with a relatively pale but definitely acidophilic cytoplasm. They range in size from 15 to 25 microns, averaging about 20 microns. The cells resemble epithelial cells. The nuclei are large, spherical and vesicular, containing one or more nucleoli with but little chromatin. Some cells contain two nucleoli. The depth of

Aschoff and Kohn. de Winiwarter introduced the term "cellules pheochromes." There seems little doubt from the embryologic researches of these authors, that the chromaffin tissue, after separating from the sympathico-chromaffin anlage, may come into close proximity to the Wolffian body and may even be incorporated with it. Later, as development proceeds, the relationship to the paroöphoron and paradidymis may become very close. When the gonads descend from their site of origin into the pelvis or scrotum, the enmeshed chromaffin cells, along with the other structures, may be carried downward and come to rest in new positions. Zuckerkandl,¹² as well as Aschoff and Kohn, has shown that chromaffin cells may be so deposited in fetuses and in the new-born as to occur along the courses of the ureters, in the broad ligaments, in the retroperitoneal tissue, in the prostate glands and elsewhere. Rieländer⁷ in several instances found chromaffin cells in relation to the paroöphoron of fetuses and the new-born. Soulié,⁹ in his extensive researches on the development of the suprarenal glands, pointed out the possibility of the occurrence of isolated or accessory chromaffin bodies. The chromaffin reaction of the cells in fetuses and in the new-born has been positive, in contrast to the negative results obtained by Berger in adult tissues. de Winiwarter has emphasized the close association of these cells with the sympathetic or non-medullated nerves of the ovaries of embryos and fetuses, a condition similar to that found in adults.

OBSERVATIONS ON THE CELLS OCCURRING IN THE OVARIES

The observations of the writer have been primarily concerned with the cells under discussion as found in the ovaries of adults. Seldom were the sympathicotrophic cells found in both ovaries. In no instance could one detect the groups or clusters of cells with the naked eye. These foci are almost always in the hila of the ovaries, lying high up in the mesovarium but generally outside of the ovarian tissue proper. Occasionally, large and small groups of the cells are found in the ovarian cortex or medulla, but in such instances they are very near the hilum. The groups of cells frequently seem to be more numerous near the poles of the ovaries, but are found throughout the entire hilum.

The relation to the large or small non-medullated nerves of the ovarian hilum is a very constant and rather characteristic feature.

One could not see the clusters of cells in the fresh tissue and consequently it was an exceedingly difficult task to locate proper material for special technic. The occurrence of these cells is by no means constant. By laborious searches, thin pieces from six different ovaries were secured and tested for their chromaphil reaction. This tissue was fixed from six to twelve hours in a five per cent solution of sodium bichromate and five per cent formalin. This solution was then drained off and the tissue preserved twelve to eighteen hours in a five or ten per cent solution of formalin. Frozen sections were made of this fixed tissue, and counterstained with a weak solution of carbol thionin. Control tissue was obtained from the suprarenal glands of rabbits and guinea pigs. In every instance the medulla of the suprarenal glands is stained yellowish or brown, while the groups of large cells of the ovarian hila are unaffected by the chromic salts.

The ovarian sections from the same tissues used in the above experiments were treated with fat stains, particularly Scarlet red, and Sudan III. In every instance the majority of the cells contain fat, varying in amount from a single small globule to complete infiltration. It is impossible to be certain regarding the staining reactions of the pigments, but it is probable that they are liprochomes.

Considerable material was used in the chromaphil tests, and little was available for the argentophil tests. Here again the results were negative. The siderophil reaction was not tried.

PATHOLOGIC AND OTHER CHANGES DURING PREGNANCY

Six pairs of ovaries from pregnant women were examined, and in four cases the cells were found. Four of the individuals died as a result of a toxemia of pregnancy, and all were in the first few months of gestation. In the four cases, fat vacuoles are seen very infrequently. The cytoplasm of the cells takes a very deep eosin stain. In three of the cases this is the only change observed, but in one instance the cells appear to have undergone a marked hyperplasia and hypertrophy. In this case, the clusters of cells are unusually large and numerous and a single group of cells fills the low power field. They are only observed in the ovary opposite the one containing the true corpus luteum and the close relationship they bear with the non-medullated nerves as well as the numerous and large lymphatics

staining of the cytoplasm appears to depend largely upon the fat content of the cells. In the cells containing much fat the vacuolated cytoplasm takes very little eosin, while other cells, but generally in a different ovary, have a deeper stain and fewer fat vacuoles. The cell membranes are clearly defined and delicate in properly preserved and fresh material. Among these groups of cells one very often finds scattered eosinophilic and mast cells, the significance of which is obscure.

In certain cells of a cluster, and very often in most of the cells of a given group, one may distinguish a few small and irregular pigment granules, brownish in color and frequently very dark. In some preparations one finds crystals in the cells. The crystals vary a great deal in size, some being short and others long, but they are generally broad with blunt ends and take the eosin stain. Very often the crystals present a clear streak or cleft running along the longitudinal axis, while a few others present sharp and ragged ends. In a few of the cells that contain one, two or more large crystals, the nuclei have become shriveled and pushed to one side. The exact nature of the crystals and granules is not known. The writer did not find these intracellular structures to be as numerous as the literature might lead one to believe, for many clusters of cells did not contain a single crystal.

The cells, at first glance, present a superficial resemblance to lutein cells, and certain persons viewing them have made this comment. They present a certain morphologic resemblance to chromaffin cells, and the close association with the non-medullated nerves, as well as the data obtained from embryologic, foetal and new-born material, strengthens this assumption. On the other hand, not a single ganglion cell is observed among the clusters of cells of the various sections.

The morphologic appearance of the cells is the same in all specimens examined, whether the ovaries were those of an infant, a young adult or an aged woman. One of the largest collections of cells found was in an ovary of a woman 60 years of age.

Because of the similarity of the sympathicotrophic cells to chromaffin cells, and because of the embryologic grounds which favor the possible occurrence of chromaffin cells in the ovary, an effort was made to study the chromaphil and argentophil reactions. In this connection it is well to emphasize the technical difficulties involved.

OBSERVATIONS ON THE CELLS OCCURRING IN THE TESTES

Undoubtedly, the same cells occur in the hila of the testicles of adults. The testicular material, however, was not abundant and was limited to six positive specimens. The cells occur in or about the non-medullated nerves of the testicular hilum, particularly near the epididymis. In none of the six testicles are the cells especially numerous, usually not more than twenty-five to thirty cells being seen in any section. In morphology, they are identical with the cells described in the ovaries, though granules and crystals are not seen. None of this tissue was subjected to special technic, because of the accidental nature of the findings.

Since the testicular hilum includes so much loose tissue, it was very difficult to examine it all, and it was probable that many groups of these cells were not found.

DISCUSSION

There is a question as to the exact nature of the cells described. The close association of the groups of cells with the non-medullated nerves of the ovarian and testicular hila was pointed out particularly by Berger. Berger called the cells "glande sympathicotrope" and regarded them as being homologous with the interstitial cells of the testis. He believed the cells arose from the nerve sheaths. Bucura considered them as chromaffin cells. Lewin called them "interstitial gland cells." de Winiwarter believed his "cellules pheochromes" of fetuses and embryos were the same as Berger's "glande sympathicotrope."

It is clear that the cell groups are not accessory chromaffin bodies, since the chromaphil, argentaphil and sideraphil reactions are negative. The three reactions were tested by Berger and the first two by the writer.

From a study of fetal and embryologic material, there are good reasons for expecting chromaffin cells in the ovaries and testes of adults, particularly in relation to the nerves. On the other hand, it is well known that chromaffin tissue undergoes regressive changes shortly after birth, and the common occurrence of this tissue in intra-uterine life is no assurance of its persistence in later years. The relationship with the non-medullated nerves is a strong point in favor of their chromaffin nature; but not in a single instance, aside from

about certain of the masses of cells are striking features. An occasional collection of these cells is found within the ovarian stroma. The abundance of the cells, as compared with the previous specimens studied, suggests that the increase is due to hyperplasia. Many of the cells are very large, in fact a third to a half larger than those found under ordinary circumstances, probably due to hypertrophy. The irregular granules mentioned above are seen in only a few of the cells, but crystals are absent. Most of the nuclei are vesicular. Crystals were not observed in any of the specimens from pregnant women; however, they were not constant during the non-gravid state.

It is also of interest to note certain other pathologic changes in the sympathicotropic cells of this individual. The woman died from pernicious vomiting in the third month of pregnancy and the corpus luteum was found to have undergone coagulation necrosis. This phase of the case has been reported by Brannan and Cohen.* The cells in question were not mentioned by them, but reserved for this paper. The illustrations show numerous pyknotic and a few shriveled nuclei (Figs. 8 to 12). Other cells present granular disintegration of their cytoplasm, and adjacent areas show a complete loss of cells, no doubt the result of necrosis. Apparently some of the sympathicotropic cells have undergone degenerative changes and necrosis as did the cells of the corpus luteum. Leucocytes and wandering cells, however, are not observed, but they are not numerous in the necrotic corpus luteum of the opposite ovary.

In this connection, it is perhaps well to mention that the well known interstitial cells of the ovary, occurring about the atretic follicles during pregnancy, were found in both ovaries of this individual, as well as in the ovaries of two of the other cases of pregnancy showing sympathicotropic cells. The staining reactions of the two groups of cells are somewhat similar, but otherwise the cells are quite different in size, position and other characteristics. No confusion in differentiation should arise between the two sets of cells, especially when they can be observed in the same section.

* Brannan, D., and Cohen, M. Necrosis of corpus luteum of pregnancy. *Surg. Gynec. Obst.*, 1926, xlii, 228.

vessels, one might expect an outpouring of some secretion into these vessels. Pregnancy has some influence on the cells as was shown by a change in their staining reaction, loss of fat and occasional hyperplasia and hypertrophy. The writer cannot subscribe to Lewin's ideas regarding function, and the cells have nothing to do with senile psychosis.

A few points are recorded concerning the histopathology of the cells when occurring in a case of pernicious vomiting showing necrosis of the corpus luteum. It is well to regard the hyperplasia and hypertrophy during gestation as a pathologic change, since it is not a constant feature. Berger also found an overgrowth of the sympathetic cells in some of the ovaries of pregnant women. It is possible that tumors may arise from these cells, particularly in the ovaries.

It is to be hoped that others will search for these elements in the gonads, so that before long we may have a better understanding of this obscure group of cells.

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the observation of Bucura, have ganglion cells been found. From a morphologic point of view they have certain features of chromaffin cells, but as pointed out above, they lack the biologic properties of chromaffin cells in adult tissues. Either Aschoff, Kohn, de Winiwarter and Rieländer were discussing entirely different cells, or the cells in question were chromaffin cells which in adults have lost their chrome-affinity, sidero-affinity and argento-affinity. Such a suggestion is obviously unsound, yet de Winiwarter conceives this idea in order to harmonize his views with those of Berger. Berger, later, refuses to accept de Winiwarter's ideas: hence the matter stands, at present, unsolved. The cells appear to be epithelial in nature.

Whatever the identity of this group of cells may be, it is unfortunate that the term "interstitial cells" or "interstitial gland cells" should be applied to them. Interstitial cells of the testes and ovaries are well known, and refer to different cells in each organ. Certainly a similar term should not be applied to another group of cells in the gonads. Since Berger has proposed the new and distinctive term of "cellules" or "glande sympathicotrope" as a result of the close association or affinity of the cells with the non-medullated or sympathetic nerves, it is only fair that we retain his distinctive terminology. There is nothing confusing about his terminology which may be translated as sympathicotropic gland or cells. The word cells is more exact than gland, because in many instances only a very few cells were found, and furthermore, there was practically no suggestion of a glandular structure, even in the largest clusters of the cells.

The occurrence of the sympathicotropic cells is not a constant finding in either the ovary or testicle. They may be found in the ovaries of the new-born, throughout childhood, during adult life and in old age, and probably also in the testes of the young as well as in the testes of the old. This group of cells is obviously the same in both the ovaries and testes. The inconstant occurrence of these cells in the gonads is very suggestive of some variable structure, like chromaffin or cortical adrenal rests found elsewhere. When present, however, they should be regarded as essentially normal structures. They have nothing to do with cortical adrenal rests. Berger believes that the cells arise from the nerve sheaths, but this is unproved and questionable.

So far as the function of the sympathicotropic cells is concerned, we are totally ignorant. From the close association with the lymph

DESCRIPTION OF PLATES

PLATE 99

- FIG. 1. A camera lucida drawing of the sympathicotrophic cells of an ovarian hilum, showing morphology; observe the granules and crystals.
- FIG. 2. A camera lucida drawing of sympathicotrophic cells as found in the ovarian hilum of a new-born child. Note the close relationship of the cells with the non-medullated nerve below, and also the cell lying in the center of the nerve. An arteriole and a vein are conspicuous.
- FIG. 3. A camera lucida drawing of the sympathicotrophic cells occurring in the hilum of a testicle. The edge of the non-medullated nerve is shown above, in and about which the cells are found. Note the two small vessels supplying the cells.

PLATE 100

- FIGS. 4 and 5. Two groups of sympathicotrophic cells from the same ovarian hilum. In Fig. 5 a small non-medullated nerve courses through the cluster of cells. Note also in Fig. 4, the proximity of the lymphatic vessels at the periphery, and the blood vessels.

PLATE 101

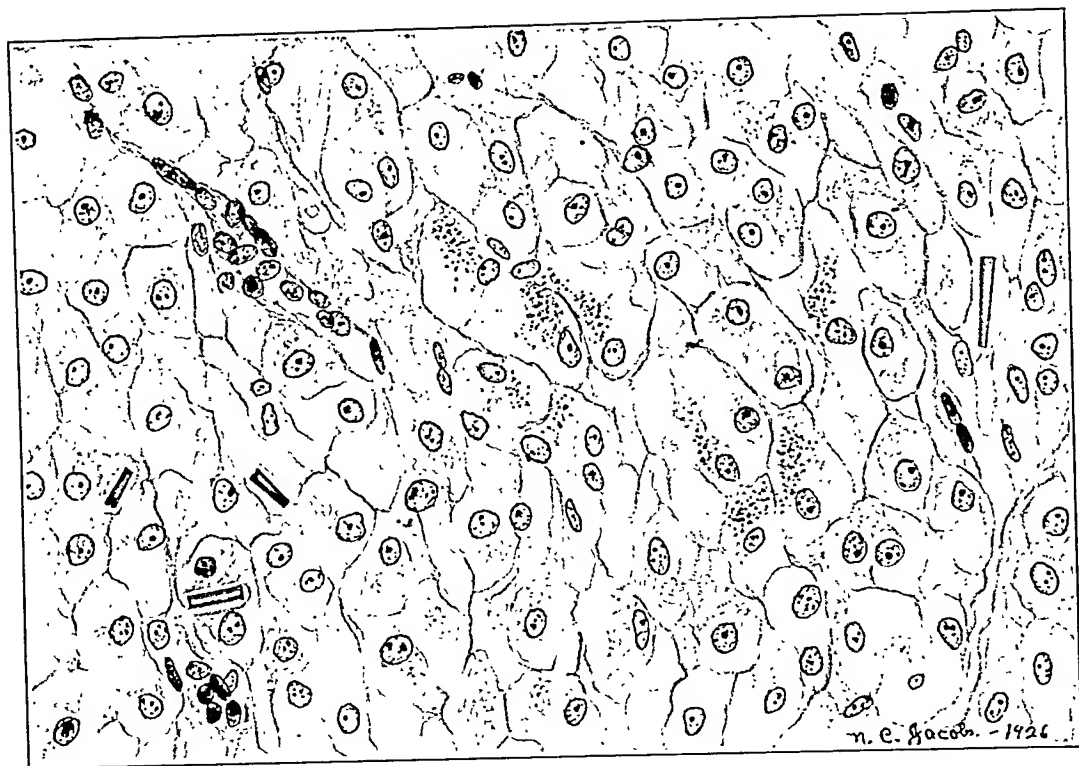
- FIG. 6. A small group of sympathicotrophic cells in an ovarian hilum near a non-medullated nerve. The lymphatic and blood vessels are conspicuous.
- FIG. 7. Another patch of sympathicotrophic cells from an ovarian hilum lying in denser tissue but along the side of a nerve.

PLATE 102

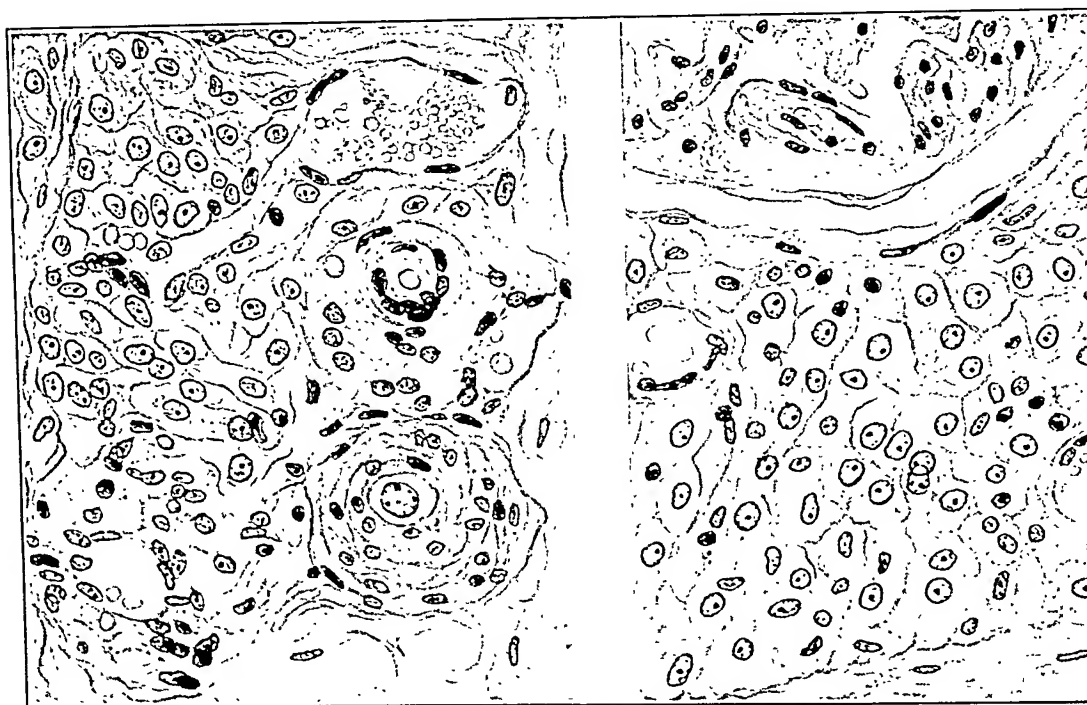
- FIGS. 8 and 9. Large hyperplastic masses of sympathicotrophic cells in the ovarian hilum from the case of pernicious vomiting of pregnancy. Note the deeper staining of the cells which occurs during pregnancy, as compared with the normal cells of the non-gravid state shown in previous illustrations. The numerous pyknotic nuclei are to be regarded as pathologic. In Fig. 8 there is a small nerve in cross-section at upper border of the group of cells. In both figures the cells are just under the peritoneal covering of the mesovarium. In Fig. 9 are also several vessels in the midst of the cell clusters and a large lymphatic running perpendicularly.

PLATE 103

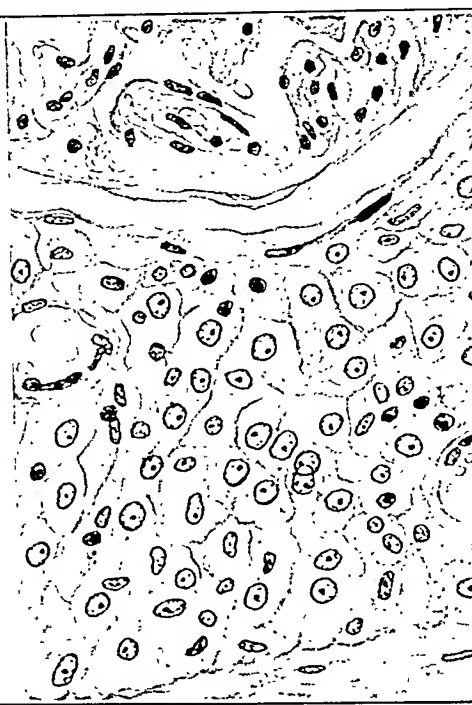
- FIG. 10. A high power magnification of the same cells from another area but from the same case of pernicious vomiting of pregnancy. There are several pyknotic nuclei and disintegrating cells, particularly in the center.
- FIG. 11. A field similar to Fig. 10. Here one can see a few granules in the cells, and one large hypertrophied cell which contains granules. There is also one polymorphonuclear leucocyte near the large cell.
- FIG. 12. Same as Figs. 10 and 11 but showing more degenerative changes. Another leucocyte is shown. The edge of a small nerve is shown above and to the right.



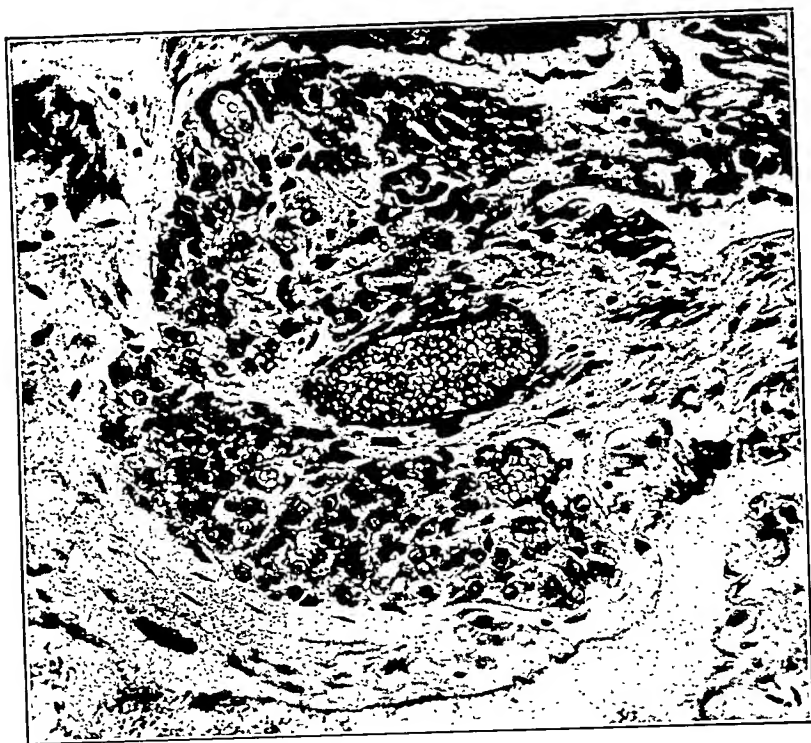
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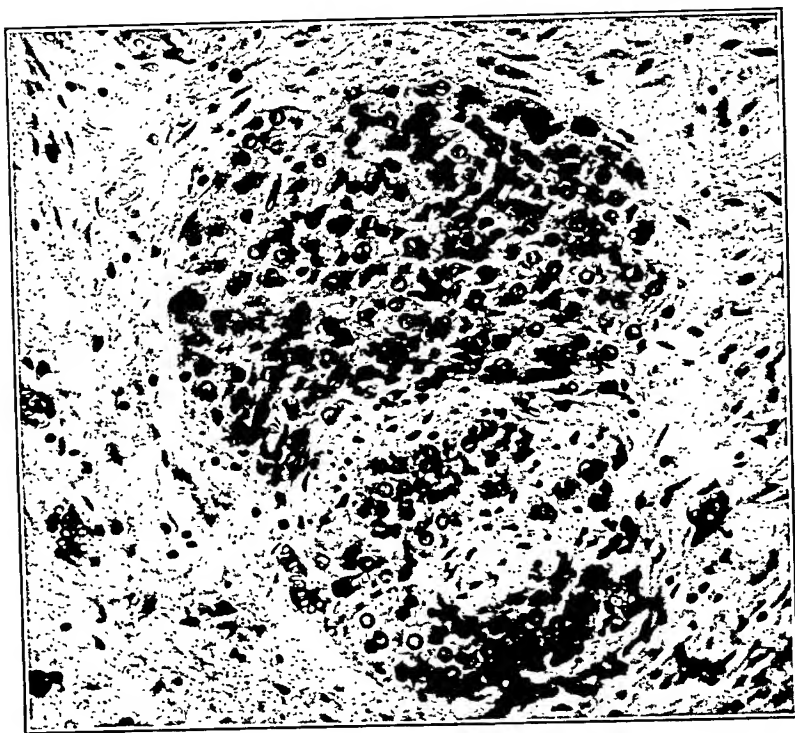
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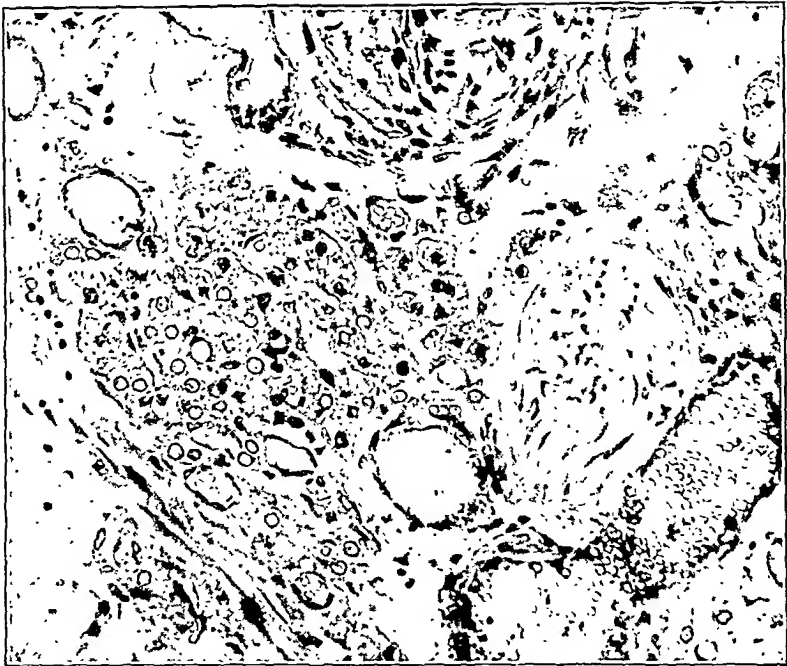
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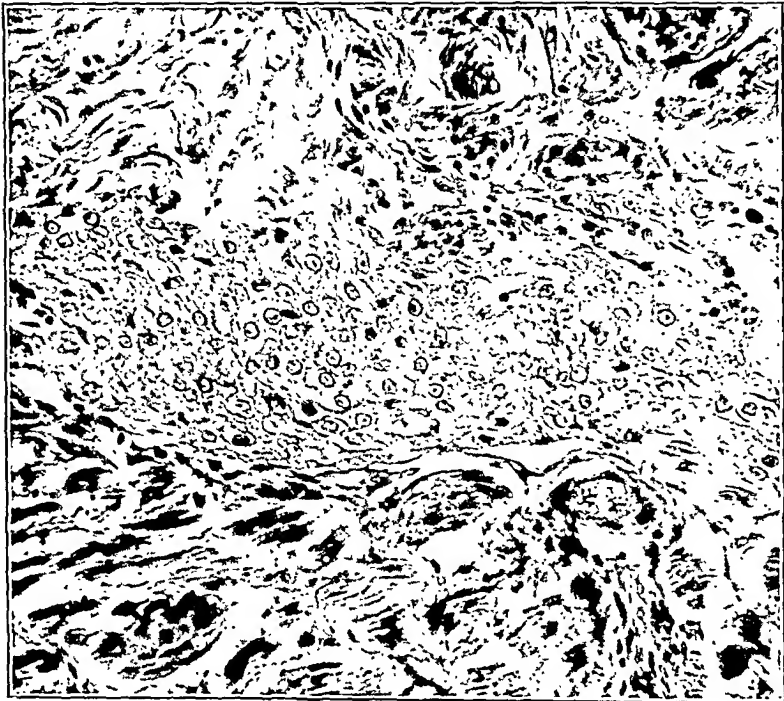
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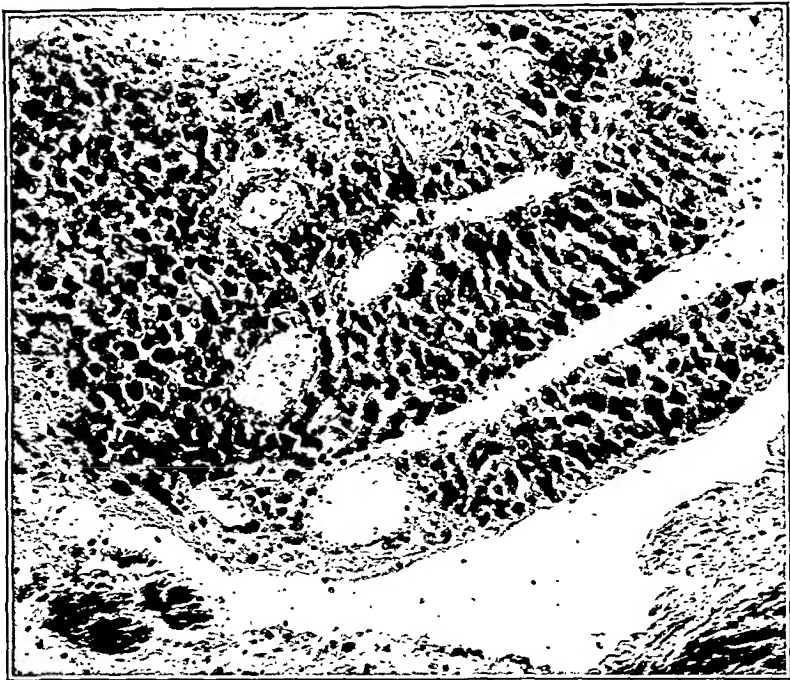
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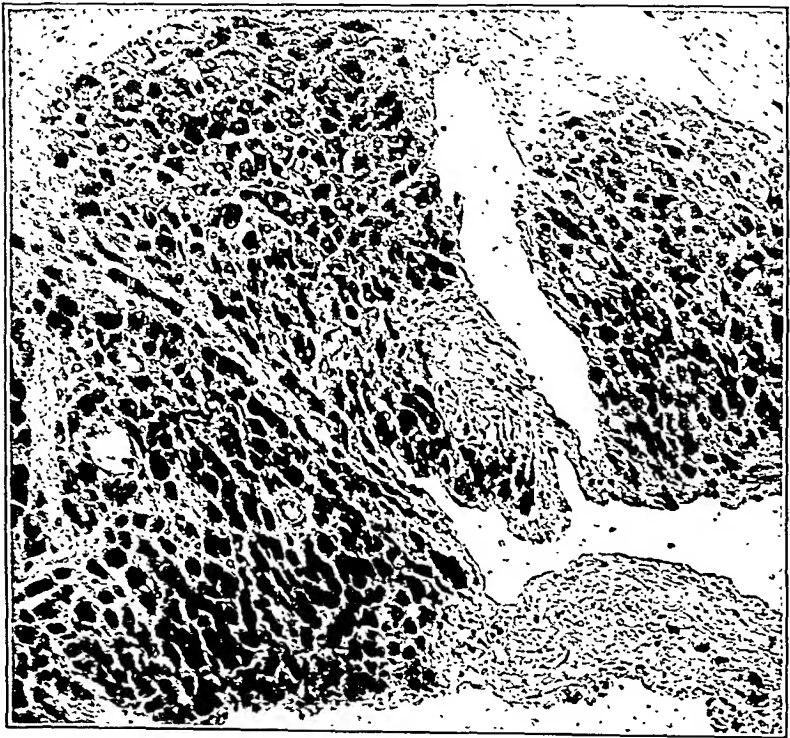
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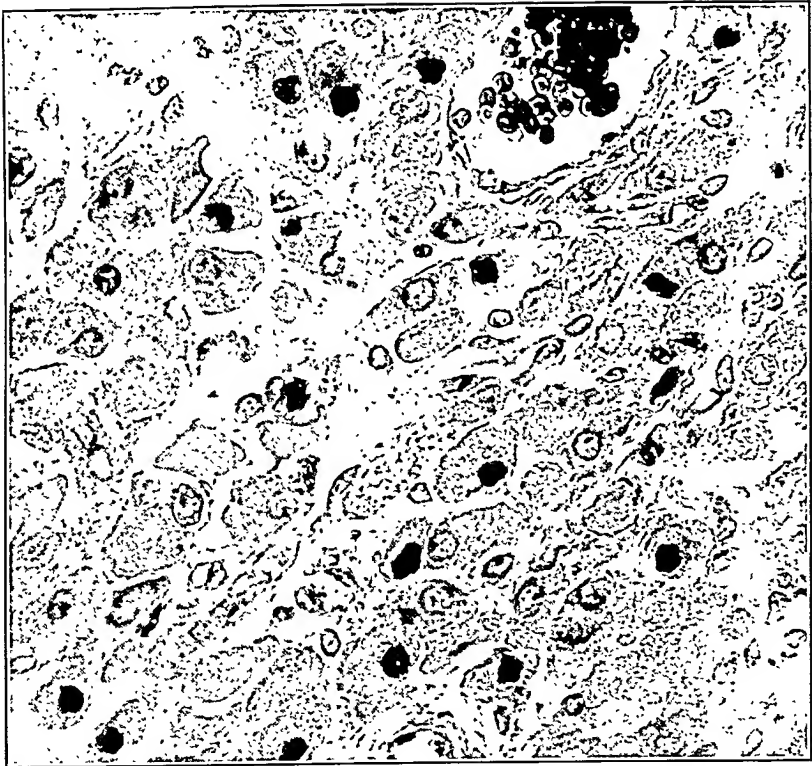
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thigh, apparently originating in relation to the fascia of Hunter's canal.

Microscopic examination: Sections stained with eosin and methylene blue, and phosphotungstic acid hematoxylin show an apparently definitely encapsulated tumor. This tumor is characterized by spaces in the form of anastomosing clefts lined in most instances by low cuboidal epithelial-like cells, and separated by compact cords of spindle-shaped cells which, in characteristic portions, seem to be devoid of intercellular substance and fibrils. There are mitotic figures in both types of cells, those lining the spaces and those composing the solid structure of the tumor. The cells lining the clefts in the preparation stained with phosphotungstic acid hematoxylin show a very delicate cuticular border, most evident in the form of terminal bars. Nevertheless, the impression is strong that these two types of cells have an identical origin. Parts of the tumor containing a small amount of fibrous tissue probably represent a stroma growth. Some of the clefts are dilated, filled with debris and cholesterol crystals. In some sections the intercellular substance is composed of a hyaline homogeneous material evidently derived from the connective tissue accompanying the growth. In cross-section this material is usually circular, in longitudinal section, elongated, giving rise to the appearance described in so-called "cylindromas."

It is impossible to assign a definite source to this growth. It agrees with the description of some endotheliomas, particularly the so-called *inter-fascial endotheliomas*. Dr. Wolbach has expressed the opinion that the tumor probably represents the type of cell lining tendon sheaths and bursae.

Subsequent history: Patient died about six months later, presumably of pulmonary metastases.

CASE No. 2. (P. B. B. H., Surgical Report No. 15615) (Pathologic Report No. S-21-813).

Abstract of clinical history: The patient is a well developed and nourished Jewess of 24 years, with a negative family and previous history. She complains of sharp, shooting pains radiating from the upper left thigh down the inner aspect of the leg to the ankle, and a mass in the upper and inner aspect of the thigh. The onset of the pain was rather insidious, beginning eight months ago. Three months before admission, the patient's sister noticed a mass in the upper left groin which has not appreciably increased in size.

Physical Examination: Negative except for an ill-defined, non-tender, hard mass 8 to 13 cm. in diameter, which extends just above Poupart's ligament and

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During the past few years there have accumulated in the pathological collection of the Harvard Medical School and the Peter Bent Brigham Hospital three rather unusual tumors, having too many points in common, both clinically and histologically, to be regarded as coincidental. In a fairly thorough search of the current medical literature of the past two decades, I have been able to find very few articles referring to similar tumors. For this reason, it is worth while calling attention to the existence of such a type of tumor. That I lay myself open to criticism in defending my thesis is apparent, but it is only by such wholesome difference of opinion that controversial points can be conclusively and ultimately settled.

The nomenclature which has been suggested in the title is a departure from the time-honored embryologic custom of designating a tumor by the type cell from which it is derived; and yet, such a term indicates obviously the tumor's origin, and conceivably offers a loophole of escape when the embryologic etiology is debatable, as in this case. Perhaps, and more correctly, one should use the term mesothelioma in this connection, for, like other serous cavities, the lining cells of the joint cavities and of the bursae are considered by most embryologists to be of mesothelial origin; or perhaps it would be more logical to utilize the much over-worked term endothelioma, and create an additional subdivision of that vast group of tumors of uncertain origin which are already included under that heading. It has seemed best, however, to classify this group with a name by which they may be easily identified, and which can readily be found in any published index. That there is a precedent for such a nomenclature is seen in the naming of other special types of tumors such as the hypernephroma, the meningioma and others.

CASE REPORTS

CASE No. 1. (P. B. B. H., Pathologic Report No. S-21-613.)

Gross specimen consists of three fragments of tissue, each measuring about 1 cm. in diameter, removed from the inner aspect of the

* Received for publication April 16, 1927.

August, 1923. Wassermann; positive. She was given 8 treatments with mercury succininide intramuscularly; however, the pain in the left leg continued. She noticed a small non-tender lump in left groin, but did not mention it at this time.

December, 1923. During the past few weeks the pain has extended down the leg to the ankle, with a drawing sensation in the groin. Appetite is good and there is no loss in weight. On physical examination, the outer aspect of the scar is tender. Palpation of the rectum reveals an elongated sharp-edged mass just outside the sphincter on the left side which feels adherent to the ascending ramus of the ischium and is slightly tender. A small mass is palpable in the right axilla.

X-ray Examination: A film of the patient shows a defect involving the ascending ramus of the ischium and descending ramus of the pubis, with several silver clips in position around this area. Bones elsewhere are normal.

June 8, 1924. The glands in the groin were excised because of recurrence.

Gross specimen: Circumscribed nodule 3 by 3 by 2 cm., surrounded by a thin fibrous capsule. It appears to be involved by tumor.

Microscopic examination: Dr. Hansman, "The nodule consists of a dense, rich, cellular tumor, made up of spindle cells with well defined nuclei, but a cell membrane is rarely seen. A few primitive blood vessels are seen as spaces lined by a single layer of endothelium, and containing red blood cells, lymphocytes and a few eosinophiles. There are numerous mitotic figures. The structure is identical with the first specimen."

March 16, 1925. *Interval history:* Patient remained entirely free from symptoms for two months, returning to the out-patient department for X-ray treatment every three weeks. In July she went to Omaha, and remained there six weeks. She had a recurrence of the same pain, two or three times a day, lasting five minutes. The left leg was slightly swollen, and she could feel several small lumps in her back just to the left and below the sacrum. Since that time she has had fairly intensive X-ray treatment, with intermittent relief of severe symptoms but persistence of transient symptoms. She was again seen in consultation by Drs. Cushing, Homans, and Sosman, all of whom advised strongly against further operative treatment and urged that X-ray therapy be given to the limit. At this time there was a large, firm, rounded mass to the left of the rectum extending from the symphysis to the sacrum, marked induration, discoloration and tenderness of the left labium majus and a small lump in the groin.

Through the courtesy of her attending physician, Dr. Thomas W. Leavitt, the subsequent history was obtained. She improved temporarily under intensive X-ray treatment but, after about two months had a further recurrence and progressively failed, dying of pulmonary metastases.

curves downward and inward over the left thigh. On rectal examination, a round, hard, non-tender mass can be palpated which invaginates the rectal wall on the left side, and is apparently connected with the mass in the thigh.

X-ray Examination, November 21: Films of the pelvis and upper two-thirds of the femur, and the shaft of the left femur show no evidence of bone involvement.

Laboratory Examinations: Blood Wassermann; positive. On December 7, the patient was shown to members of the society of Clinical Surgery by Dr. Harvey Cushing, as a probable case of a semibenign growth of the nature of the desmoid tumors of Nélaton, although he was aware that such tumors are usually found in the abdominal wall.

Operation Report: Dr. Harvey Cushing, December 7. "Surface of tumor completely covered by the adductor group; the fibers split until the surface of what was evidently an enucleable, well encapsulated growth encountered. Surface was vascular and so elastic as to give impression of abscess or cyst. Growth large as two fists, many layers and bands of tissue over it. Tumor itself not vascular; contained many small cysts."

Pathologic report: The tumor is an unusual one, composed of spindle cells grouped in anastomosing cords and separated by endothelial-lined clefts. This is the typical arrangement of the tumor although large areas are seen where the growth is compact but in which new vascular channels although compressed can be made out. There are some areas where the tissue is compact and contains a small amount of collagen which is probably derived from an ingrowth of connective tissue cells. Mitotic figures are fairly numerous.

COMMENT: The tumor is one on which absolute classification remains suspended. The general arrangement is not wholly incompatible with a tumor of connective tissue origin, but the absence of fibrils and intracellular substance is against this diagnosis. There are similar tumors described as endotheliomas, and their origin attributed to fascia. The intimate relation of cell columns to endothelial-lined blood-containing spaces supports the diagnosis of endothelioma. Similar arrangements of tumor cells are found in solid tumors of the ovary, and it must be borne in mind that solid tumors of the ovary, regarded as epithelial in origin, are often composed of spindle-shaped cells. The possibility of the tumor originating in the pelvis from ovarian tissue must be borne in mind.

May 21, 1923. *Interval history*: Patient was well for two months after leaving the hospital, when she again began to have occasional sharp shooting pains in the left thigh. These have increased in frequency and severity during the past ten months.

Pathologic report: II: The specimen consists of a leg amputated 15 cm. above the knee joint. The knee is swollen for some distance both above and below the patella, and in addition there is a definite tumor nodule on the lateral aspect of the knee just above the patella, which measures 4 to 6 cm. in diameter, and is elevated 2.5 cm. above the general surface. This has practically broken through the skin in one or two places. It is discolored by hemorrhage and necrosis. Two other smaller subcutaneous nodules are noted around the patella, the larger of these measuring 1 cm. in diameter, and being slightly elevated. The specimen is split longitudinally through the knee joint. The entire joint cavity is filled with tumor tissue which has infiltrated the patella, the tibia and the femur so that these bones around the knee joint can readily be cut with a heavy knife. The tumor is made up of very soft grayish friable tissue which grossly has no very definite structure or stroma. It is comparatively avascular. It is not unlike the appearance of a lymphoid or a neuroblastic round cell sarcoma in its consistence, but does not resemble it in other respects. The tumor involves the surrounding structures, including the fascia, muscles and subcutaneous fat. It has caused intense pressure on the sciatic nerve, which presumably accounts for the clinical pain.

Microscopic examination: Further histologic sections of the specimen present essentially the same characteristics as noted in the earlier specimen except that the spindle form of the cells is more uniformly present. Numerous mitoses and occasionally multiple mitoses are encountered. It suggests a very rapidly growing sarcoma, probably of synovial membrane origin.

DISCUSSION

In reviewing the literature, two papers of particular value in respect to this group of tumors have appeared in the past few years; a brief discussion of the classification of the tumors of the knee joint by Züllig,¹⁸ and a review of the reported cases up to 1923 by Faccini.⁵

clined to think that it was a fibrosarcoma with an inflammatory hyperplasia of the synovial membrane attempting to wall off the tumor, and felt that the prognosis was dependent on its connective tissue metastatic character. Dr. S. B. Wolbach concurred more definitely with me in the feeling that the tumor presumably arose from the synovial membrane, as both Wright and Ewing tacitly imply by their diagnosis of endothelioma.

CASE No. 3. (Pathologic Reports Nos. H-24-30, H-25-296.)

This case is presented through the courtesy of the attending surgeon, Dr. James S. Stone.

Clinical history: The patient was a man 35 years of age, who had a swelling on the inside of the knee for about five months before admission to the hospital. This appeared to be under the vastus internus muscle, suggesting an origin from the synovial membrane. It did not appear to be connected with the bone. It was semifluctuant in places, but for the most part was fairly solid in consistence. The first operation consisted of an excision of the tumor mass with the surrounding tissues. The patient remained fairly well for a number of months, when a recurrence of the lesion was noted. He was treated by X-ray and radium for a period, without marked improvement. Fifteen months following the original operation, the leg was amputated 15 cm. above the knee joint. No nodes were palpable in the groin at this time. He showed, however, at the time of operation, definite metastases in the lung by X-ray, and proceeded to fail slowly during the next five months, dying two and a half years after the first appearance of the lesion.

Pathologic report: I: The original specimen consisted of a tumor mass measuring 8 by 6 by 3 cm., with considerable thickened synovial membrane and fascia attached. The tumor at one point contained a hard calcified irregularly outlined mass 3 cm. in its greatest extent. On section the tumor had a fairly well defined capsule which was extremely thickened. Centrally there were several foci of hemorrhage and necrosis, and cystic areas lined by smooth glistening walls. There was no gross evidence of invasion of this thickened synovial capsule.

Microscopic examination: Slides show a rapidly growing tumor with many mitoses. There is histologic evidence of infiltration of the capsule and the surrounding stroma. It is an unusual type of tumor microscopically, as the cells show a dual differentiation; some of them apparently forming synovial membrane, and resembling endothelial or almost epithelial cells, while the stroma is composed of the connective tissue type of cell. An exact diagnosis is not easily made, but a tentative one of mesothelioma or endothelioma is suggested. In view of the results in the other two similar cases which we have on record, the prognosis is presumably poor. Probably metastases have already occurred and for that reason amputation seems futile. Intensive radiation would seem to be the most logical form of treatment.*

* Personal communications. The sections were submitted to a number of pathologists and no absolute concurrence in diagnosis was made. Drs. J. Homer Wright and James Ewing made a tentative diagnosis of endothelioma. Dr. F. B. Mallory was in-

from the lining of the joint capsule and from the bursae, are essentially the same genetically, and differ only in their physical distribution. Clinically, there occasionally occur other tumors which may be confused with these groups. Of these, the rare cases of hemangioma of the joint may be cited. A recent case of this type was reported by Osgood.¹⁴ Similarly, the fatty tumors of the capsule have frequently given rise to difficulties in differential diagnosis, particularly the type *lipoma arborescens*, which Züllig¹⁸ discusses. And, finally, there are the sarcomas of fibroblastic origin, either in their simple fibrous tissue form, or differential chondroblastic or osteogenic forms. These are too numerous to cite in a paper which is limited, as this is. The essential pathology of these tumors has been brought out by many of the papers recorded above.

A recent paper by Zeckwer¹⁷ from this department, has emphasized particularly the extreme variability of the cytology of this group of tumors derived from mesothelium. In the case which she presented of a tumor originating in the pleura, three distinct cell types were found, both in their fully differentiated forms and in their intermediate forms, which could be traced back to a common multipotential cell. This is obviously what one might expect in such tumors, in view of their origin from such a relatively undifferentiated cell type, and yet it is a fact which seems to have been overlooked or neglected by most of the contributors to this subject. In the three cases which are being presented, these same features of differentiation into two kinds of cells, the synovial lining type and the supporting stromal type, with intermediate forms between the two, are seen. This variation is illustrated by the drawings and photomicrographs appended. Particularly interesting is the point which she has made of the method by which lumina appear to develop as the result of vacuolization of the cytoplasm of certain of the cells which gradually become lined by additional cells formed by mitoses from the nucleus of the original cell. Similarly, the formation of pseudogiant cells in this manner is readily explained. By what influence the differentiation of the cell from the pure spindle-appearing type to the obvious endothelial cuboidal type is brought about is extremely difficult to explain.

The latter author refers to a monograph on tumors published by Barbacci¹ in 1915, in which several examples of this group of tumors allied to this type were recorded. Unfortunately, this volume has not been available, and the references as given by Faccini of the individual cases were inadequate for verification. In general, the tumors may be said to arise as follows:

1. In relation to the synovial membrane of the joint cavity itself; such as those reported by Rijssel,¹⁵ Marsh,¹¹ Lockwood⁹ and Faccini.⁵

2. From the synovial membrane of some of the overlying bursae, such as that reported by Smirnoff.¹⁶

3. From the fascial aponeurosis, of which a rather special variety seems to be the group occurring in relation to the rectus abdominis aponeurosis, and to which the same *desmoid* was given by Müller,¹³ and which has been subsequently used quite generally. In addition to this rather limited type, the general term *fascial endotheliomata* has been applied by Ewing.⁴ Gobbi⁶ and Bolognesi² have reported typical tumors of this general variety.

4. Tumors arising in relation to the tendons and tendon sheaths. Buxton,³ in reviewing the literature of this group of tumors, comes to the conclusion that there are probably no true primary tumors of the tendon itself, that the giant cell myeloma is the commonest tumor involving the tendon sheath, and that clinically they are particularly confused with simple inflammatory processes of the tendon sheath. McWhorter and Weeks¹² subsequently discuss the rather special group of tumors of the tendon sheaths usually described by the name *xanthoma*. They conclude that *xanthoma tuberosum multiplex* and all forms of xanthomas are the result of a systemic disease in which hypercholesterolemia is an essential feature. They feel that these nodules are not, strictly speaking, tumors, but are the result of an irritative connective tissue reaction to the deposition of cholesterin; and that, as recurrence follows the surgical removal of these nodules, surgery is indicated only in cases with pressure symptoms, because surgery does not affect the underlying etiologic process.

Accordingly, there is some relationship among the tumors of these various groups, although the last division of the xanthomas suggests an inflammatory reaction rather more definitely than do the others. Certain it is that the first two groups, the tumors arising directly

DESCRIPTION OF PLATES

PLATE 104

Case I

FIGS 1 AND 2. High power photomicrographs from different parts of tumor, illustrating multipotential differentiation, in Fig. 1, the spindle cell predominating; in Fig. 2 the synovial form well defined.

PLATE 105

Case II

FIG. 3. High power. Illustrates alveolar formation about fatty secretion.

FIG. 4. Oil immersion. Illustrates polypotentiality of cells, differentiating in both synovial and fibrous fashion.

PLATE 106

Case III

FIG. 5. Low power photomicrograph. Shows extent to which differentiation may be carried in these tumors, with development of well formed synovial lining cells.

FIG. 6. Oil immersion. Same.

PLATE 107

Case III

FIG. 7. Low power camera lucida drawing. Illustrates the dual differentiation of the cells into synovial and fibrous tissue.

FIG. 8. High power camera lucida drawing. Illustrates the common parentage of the cells. Note mitotic figure centrally. Shows tendency of alveolar development from "signet ring." Vacuolization of cells.

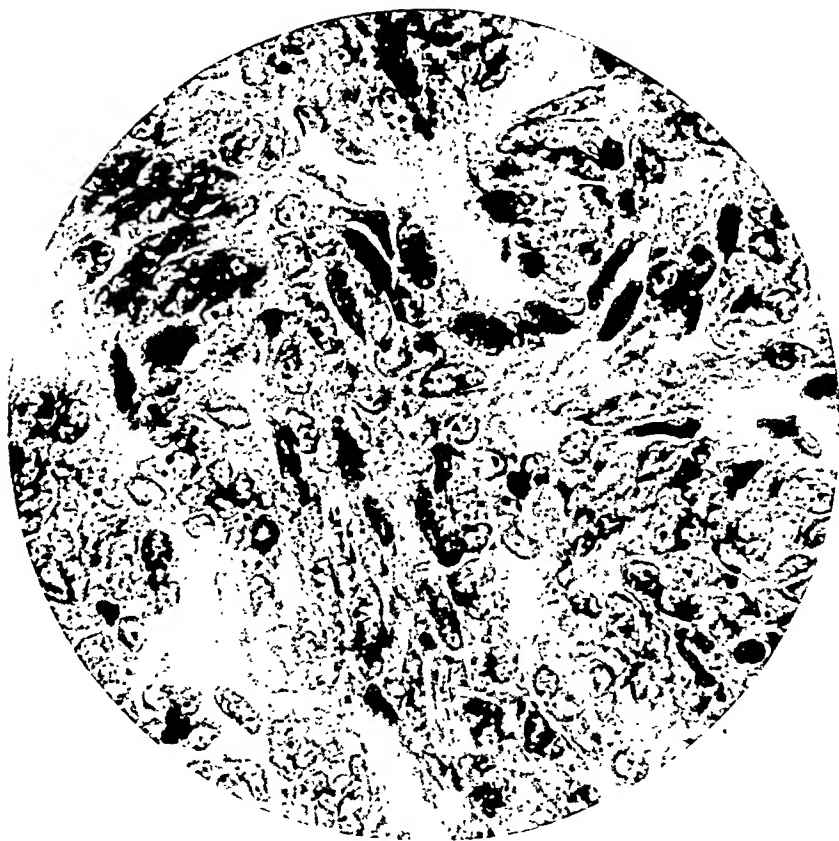
SUMMARY

Three tumors of synovial membrane origin and presenting too many points in common, both clinically and histologically, to be regarded as coincidental, are presented as a type tumor. Their histology, as based on their embryologic origin from mesothelium, is discussed, the type cell showing multipotential characteristics comparable to other mesothelial tumors.

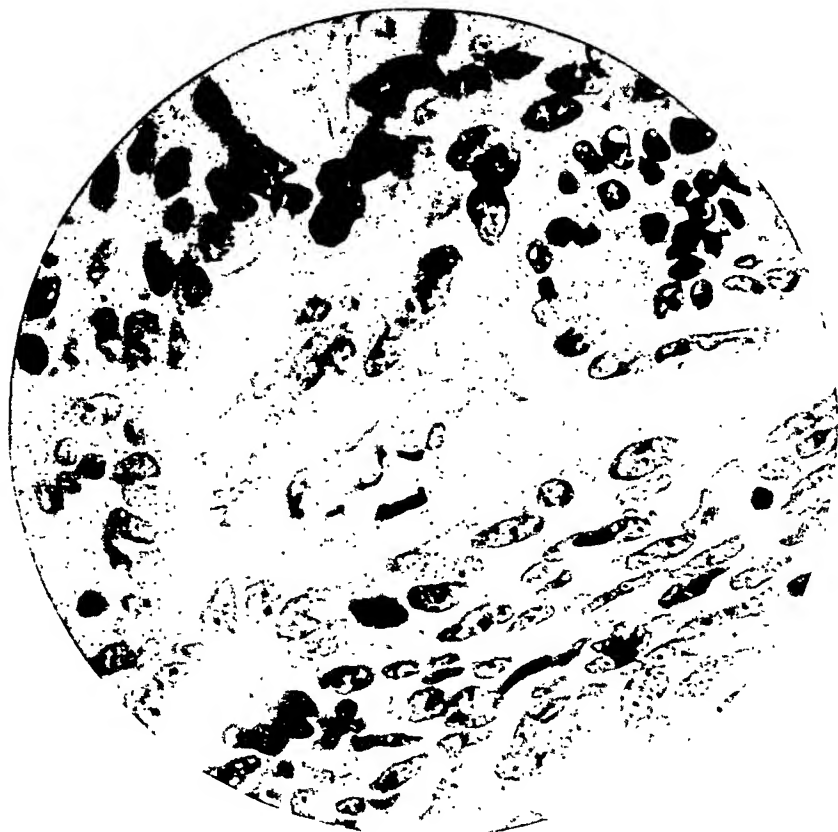
A review of the literature of the subject is presented.

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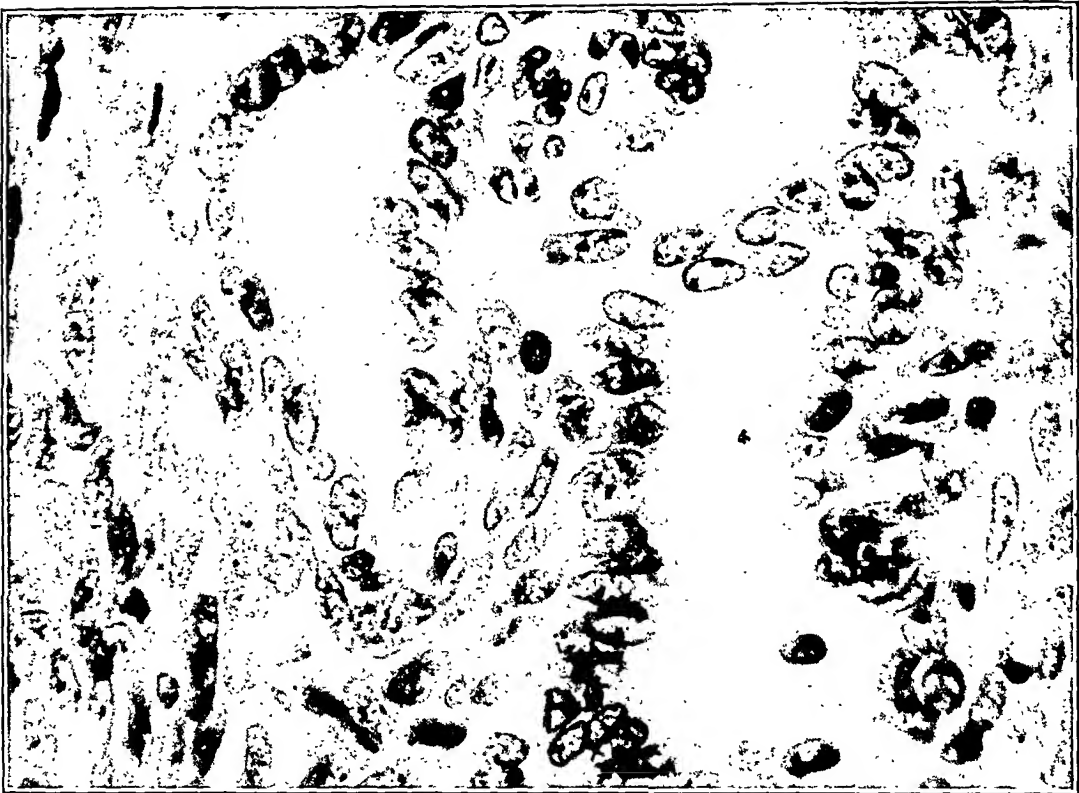


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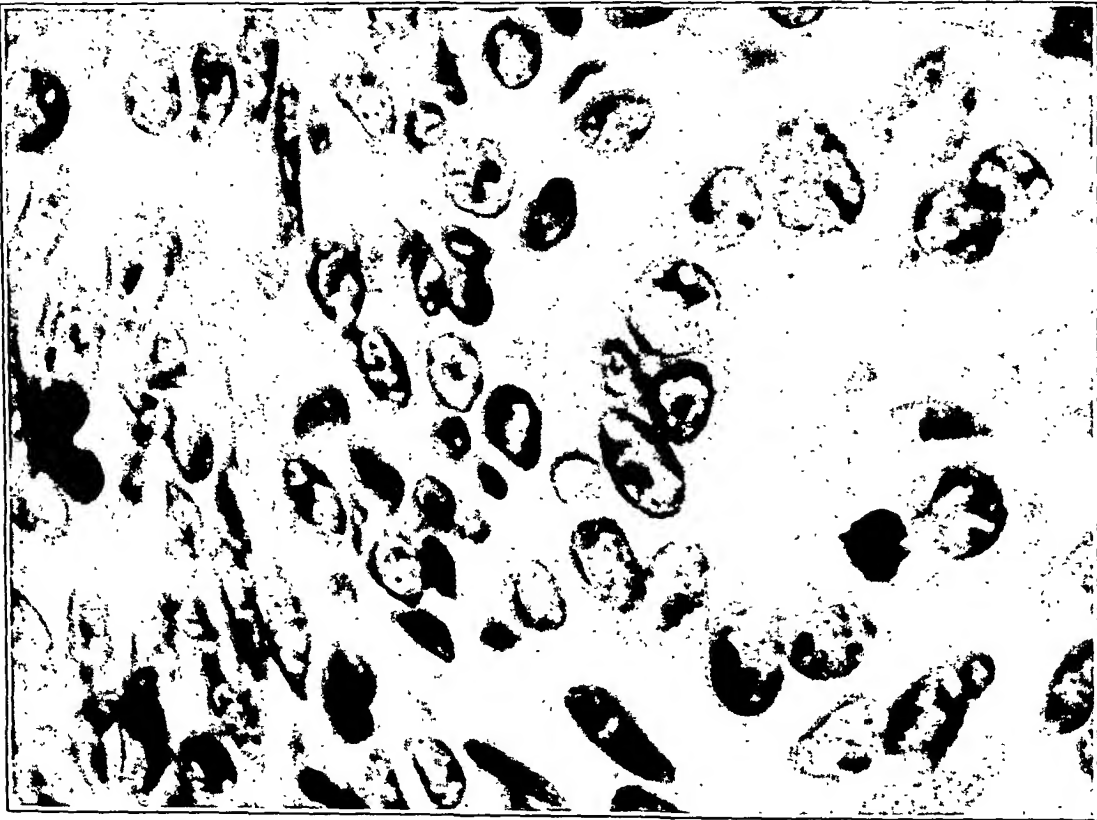


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Synoviomata



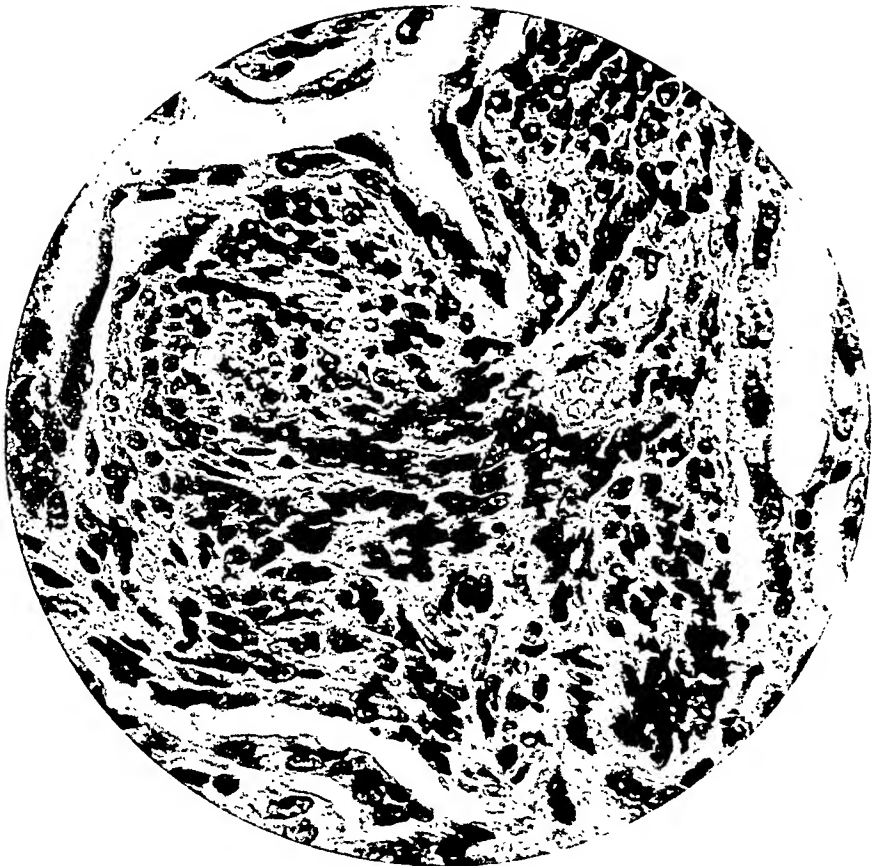
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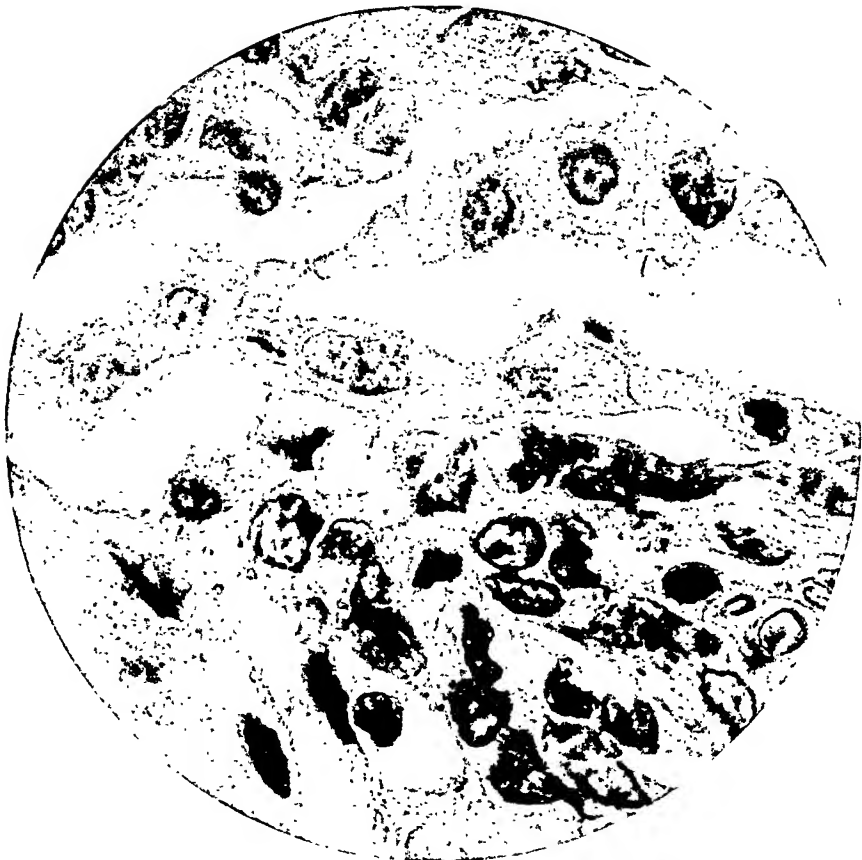
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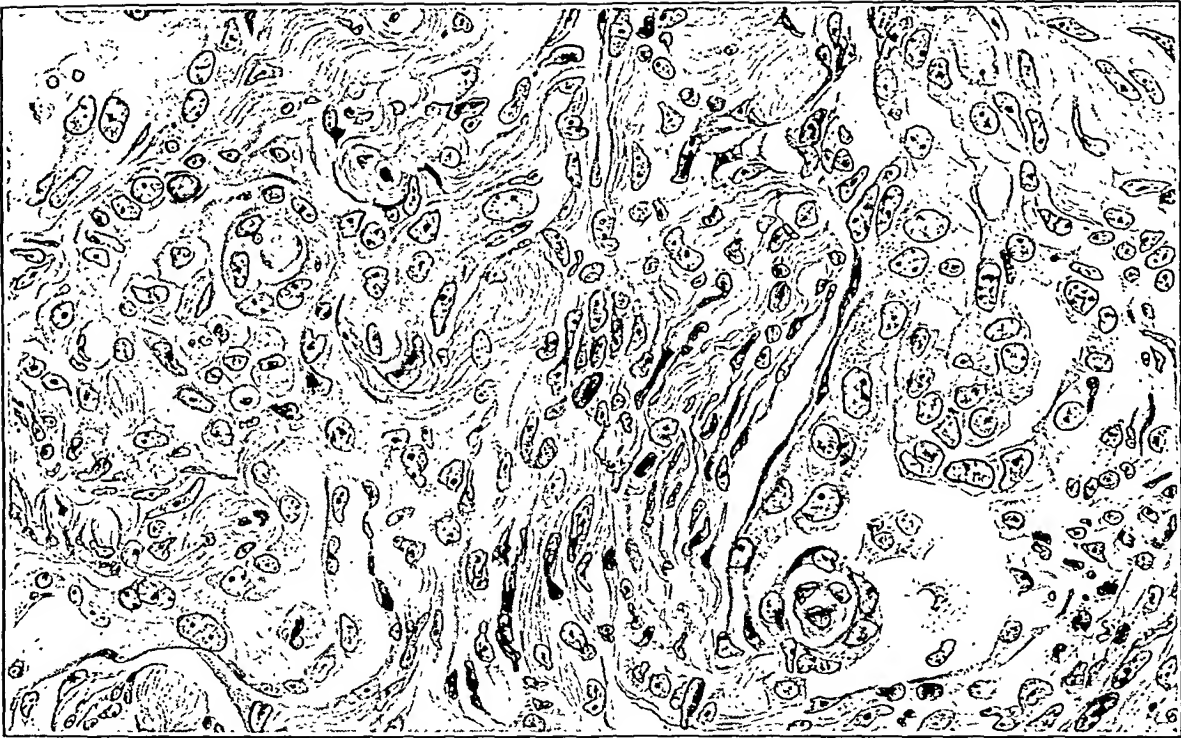
Synoviomata



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While the histologic and the accompanying mitochondrial changes occurred in the organ, there was no detectable evidence of variations in the functional activity of the gland. Also, in a few human glands examined at that time, similar variations in the number, form and distribution of the mitochondria were found to be dependent upon the size variations of the cells, but could not be correlated with the clinical symptoms.

Goetsch's findings, which led to the belief that variations in the mitochondria of the thyroid were associated with clinical symptoms, were based upon studies of human glands from cases of the so-called "toxic" adenoma. For our experimental studies, we used the common laboratory animals in whose glands adenomas were not encountered. In the previous communication, we attempted to explain the different results of the animal and human studies on the basis that in the adenomas the mitochondrial response to iodine variations might be unlike that in non-adenomatous tissues, since Marine had shown that histologically the cells in the adenomas do not react quantitatively like the cells in the non-adenomatous tissue. That is, some cells did not react at all, others very slowly and still others equally as well as in normal tissue. At that time, it was also inferred that any slight peculiarities in the iodine response of the mitochondria of the cells of the adenomas would not be associated with the clinical symptoms since the wide variations in the mitochondrial contents of the cells of normal tissues were not associated with detectable alterations in the functional activity of the gland.

An investigation of the mitochondria in the human gland was undertaken in order to determine whether mitochondrial alterations, unlike those which had been observed in the cells of non-adenomatous tissue, occurred in the adenomas whose reaction to iodine is variable; and to inquire further into the possible correlation of the mitochondrial alterations with the clinical symptoms of thyroid disease.

A study of thirty-one human glands confirms our previous findings in animals, namely that specific alterations in the number, form and distribution of the mitochondria depend upon variations in the *size* of the cell, both in adenomas as well as normal tissue, and cannot be correlated with clinical symptoms. However, during the study of these specimens our attention was attracted to variable numbers of cells in which the mitochondrial content is markedly

STUDIES ON MITOCHONDRIA *

II. THE OCCURRENCE OF MITOCHONDRIA-RICH AND MITOCHONDRIA-POOR CELLS IN THE THYROID GLAND OF MAN AND ANIMALS

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The rôle of mitochondria in cellular activities is unknown. In the progress of a systematic study of this subject, the fact that the histologic variations of the thyroid are experimentally controllable led us to pay special attention to this organ, in the belief that alterations of the mitochondria, if there were any, might be better evaluated in the thyroid cell than in the cells of other tissues. A study of the thyroid cell appeared all the more appropriate for investigating the possibility that mitochondria may serve as cytologic indicators of cellular activities, since Goetsch¹ had reported a correlation of variations in the functional activity of the gland, as detectable by clinical symptoms, with alterations in the mitochondria of the cells.

Experimentally² it was found that definite alterations in the number, form and distribution of the mitochondria in the thyroid cell were associated with the general histologic alterations which, as Marine has shown, depend upon variations in the percentage-iodine content of the organ. The hypertrophic, tall, columnar cell invariably contained innumerable, filamentous types oriented parallel with the long axis of the cell, and large accumulations near the lumen border. As the cell became flatter the filaments became fewer; rod-like and granular forms appeared, and the apical masses disappeared. In the extremely flattened cells of the completely involuted gland only a few, scattered granules were present in the scant cytoplasm.

In these animal experiments, the tall cells were obtained by feeding high fat diets or by producing states of compensatory hypertrophy by partial removal of the organ. The flattened cells were readily obtained by the administration of iodine both to normal animals and to those in which hypertrophy had been well established.

* Presented in abstract before the American Society for Experimental Pathology, Rochester, N. Y., April 14, 1927.

Received for publication March 27, 1927.

cells, and have undertaken experiments along various lines in an attempt to determine their significance.

After a more detailed description of these cells, we wish to present the results obtained from an attempt to correlate the clinical data and the gross anatomic changes with the presence of the mitochondria-rich cells in the human gland. We believe that although the series is small, the findings may have some bearing on the results obtained by Goetsch, and may serve to shed some light on the possible significance of these cells.

MATERIAL

In addition to the human glands, the thyroids of several hundred animals, including guinea-pigs, rabbits, rats, cats, dogs, chickens and pigeons were examined. For the human specimens we are indebted to Dr. George Crile of the Cleveland Clinic and Dr. Benjamin S. Kline of the Mount Sinai Hospital, Cleveland. For some of the differential staining properties of the mitochondria-rich cells we used numerous specimens which were obtained at necropsy, and had been fixed in various solutions for a period varying from a few days to several years. Many enlarged glands of dogs were obtained through the kindness of Dr. Julius M. Rogoff of Cleveland and Dr. S. E. Sanderson of Detroit. The remainder of the animal material consisted of the specimens which had formed the basis for our previous studies, supplemented by other material obtained from animals in studying mitochondrial differentiations.

METHODS

The human specimens were fixed in Regaud's solution never later than two to three minutes after removal from the body, while the animal material was fixed immediately. It might be emphasized that the blocks, although only several millimeters in thickness, were larger than are ordinarily used in cytologic studies. We attempted, wherever possible, to obtain sections almost 20 mm. square in order that in the grossly variable tissues we might be better able to control and evaluate the cytologic findings. The tissues were kept for four days in 3 per cent potassium bichromate (4 parts) and neutral concentrated formalin (1 part), changing daily; and then in 3 per cent potassium bichromate for eight days, changing every other day.

increased or decreased compared to the amounts normally present in the thyroid cell. *The variations in the amounts of mitochondrial substance in these cells are not dependent upon variations in the size of the cell.* The significance of these variations has not been determined. Before presenting the available data, it may be of interest to trace the steps which led to the detection of these mitochondria-rich and mitochondria-poor cells in the thyroid gland of man and animals.

The standardized technic employed for the demonstration of the mitochondria in the thyroid cell has been fixation in Regaud's solution, and staining with acid fuchsin and methyl green. In examining the sections upon which our previous report was based, a few deeply red-staining cells, seen in the glands of all the animals and in the few human specimens, were passed by without any special attention, in the belief that they belonged to the type of cell described by Langendorff³ as the "colloid" cell of the thyroid, since they appear singly or in small groups in a few follicles. In the glands of animals used in subsequent experiments, large numbers of cells were seen which stain paler than the average. The heavily red-staining property of certain cells is found to be due to the fact that there are larger numbers of mitochondria which are much thicker than those normally present in the thyroid cell; while the pallor of other cells is due to the paucity and the fineness of the mitochondria. Moreover, the variations of the thicker and thinner mitochondria resemble those normally present, in that they are filamentous when the cells are tall and become rod-like and granular as the cells become flatter. In the human specimens collected for this study, enormous numbers of the heavily staining cells are found scattered throughout, and frequently their positions are parietal, like those described by Bensley,⁴ as the "ovoid" cells in the thyroid of the opossum. In sections from the adenomas, these cells not only comprise the greater portions of many of the follicles but, in large areas, entire follicles are replaced by them. The pale staining or mitochondria-poor cells are relatively few in number.

The striking fact is that while both the mitochondria-rich and the mitochondria-poor cells, like the normal cells, undergo hypertrophy and involution, their numbers are not constant in the different species of animals and in the same species under different conditions. We are investigating the exact distribution among the different species of animals of the mitochondria-rich and mitochondria-poor

as to be represented by merely a distortion of the architecture and irregularity of the follicles and blood supply. In the definitely encapsulated adenomas these cells occupy large areas of the section. In the solitary large adenomas, such follicles are found beneath the hyalinized and at times calcified capsule (Fig. 6), and in the centers of the degenerating foci (Fig. 7).

(b) *Their response to iodine variations.* On the basis that the involution and hypertrophy of the thyroid cell are due to the variations in percentage-iodine content of the gland, it is a striking fact that the mitochondria-rich cells show all the variations seen in the chief cells. When they are situated in the walls of the follicles they vary from the flattened to the tall columnar types, but in the same follicle the mitochondria-rich cells may be flatter or taller than the adjacent chief cells. Adjacent follicles made up of mitochondria-rich cells may consist entirely of columnar or flattened cells. However, when they occur peripherally (like the "ovoid" cells of Bensley), or lie in the interfollicular spaces, or in the solid masses in and about the lymphoid accumulations they usually appear as giant cells varying from three to five times the size of the normal thyroid cell (Figs. 4, 6, and 7). In these locations, the shapes of these cells may be oval, round, triangular or stellate.

(c) *Their relation to the colloid content of the follicles.* Like the colloid content in follicles of chief cells, the amount varies inversely with the degree of hypertrophy of the mitochondria-rich cells, for when the cells are tall the colloid is reduced; and when the cells are flat the colloid is increased.

(d) *Their relation to lymphoid foci.* The mitochondria-rich cells are found singly and in large masses both within and at the periphery of the lymphoid foci.

(e) *Their presence within the lumina of follicles.* At times the mitochondria-rich cells lie freely within the lumina of follicles made up entirely of the same cells, at times they are contained within follicles made up entirely of chief cells; and conversely, follicles of mitochondria-rich cells may inclose chief cells (Fig. 5).

(f) *Their participation in "budding" processes.* The papillary projections into the lumina may be entirely made up of mitochondria-rich cells, or only in part, though the remainder of the follicles are composed of chief cells (Fig. 8). On the other hand, when the budding takes place toward the interfollicular spaces, the bud of

After cutting at 4 to 6 microns in paraffin, the sections were stained with acid fuchsin and methyl green according to the method given by Cowdry.⁵

OBSERVATIONS

In any tissue which has been fixed in Regaud's solution and stained with acid fuchsin and methyl green, as described above, the mitochondria are stained red, while the remainder of the cytoplasm and the nucleus are stained green. When the sections are examined with the low powers of the microscope, the cells which contain large amounts of mitochondrial substance appear deeply red stained; those containing moderate amounts appear purplish, on account of the blending of the red and green; while those containing small amounts appear uniformly green-staining.

Sections of the thyroid show all three types of cells. With the oil immersion lens, it is seen that the red-staining cells contain relatively more numerous and thicker mitochondria, while the green-staining cells contain relatively fewer and thinner mitochondria than are contained in the purple-staining or chief cells. The following descriptions present the available data concerning the chief, the mitochondria-rich and the mitochondria-poor cells.

1. **THE CHIEF CELLS.** In all the thyroids examined, the cells which appear purplish under the low powers of the microscope show constant alterations in the number, form and distribution of the mitochondria according to the degree of cellular hypertrophy or involution, as described previously² (Figs. 1 and 2).

2. **THE MITOCHONDRIA-RICH CELLS.** In the glands of the common laboratory animals these cells occur most often singly in scattered follicles, occasionally several within a follicle and rarely they replace an entire follicle (Fig. 3). In the human they are usually more numerous than in the animal glands, and in the majority of the cases studied they occupy large segments of many of the follicles and frequently replace entire follicles. They often occur in large numbers at the periphery of the follicle (like the "ovoid" cells of Bensley), in the interfollicular spaces, and occasionally they are seen within the lumen of the follicle (Figs. 4, 5, 6 and 7).

(a) *Their presence in adenomas.* Follicles made up entirely of mitochondria-rich cells occur most often in glands which show some evidence of adenomatous changes which, however, may be so slight

sition" cells is an important point to be considered when discussing the possible significance of the mitochondria-rich cells.

The fuchsin-staining affinity of the thick mitochondria in the mitochondria-rich cells is apparently the same as the finer types of mitochondria found in the chief and in the mitochondria-poor cells, but their thickness makes them appear darker staining under the same magnifications. However, incompleeted studies using various fixatives and staining methods on fresh and necropsy material seem to indicate that these thick mitochondria are chemically different from the mitochondria in the chief and mitochondria-poor cells. For the present the following may be mentioned:

- (1) After being stained with fuchsin they are not as easily decolorized by dilute acids and alcohol.
- (2) With various fixatives, they stain well with fuchsin and hematoxylin, long after postmortem effects have rendered the mitochondria in the other cells unstainable.
- (3) They are partially dissolved by lipid solvents, both before and after fixation, in a manner quantitatively and qualitatively unlike those in the chief and mitochondria-poor cells.
- (4) They are better stained with iron hematoxylin after formalin fixation, and stain poorly with acid fuchsin after fixatives containing osmic acid.
- (5) They are more refractory to those subtle, unknown technical errors which at times destroy the mitochondria in all tissues which apparently have been correctly fixed and stained, as shown by their survival in a section in which, for entirely unknown reasons, the mitochondria in the chief cells and mitochondria-poor cells do not stain.
- (6) They are stainable with acid fuchsin after refixation in Regaud's solution of tissues which had been fixed routinely in formalin, Orth's or Zenker-formol solutions, even after several years.

3. THE MITOCHONDRIA-POOR CELLS. In the glands of all animals there are cells which, when viewed with the low powers of the microscope, are uniformly green due to the relatively fewer and thinner mitochondria present as compared with those in the chief cells. The mitochondria-poor cells are present in very small numbers in the human glands, including the normal and adenomatous, but are very numerous in the thyroids of the other animals examined. There seems to be an inverse relationship between the number of mito-

mitochondria-rich cells is always found to arise from similar cells lying in the wall of the follicles.

(g) *Their nuclei.* The nuclei in the mitochondria-rich cells vary in size and staining properties as they do in the chief cells. The giant cells, however, may contain very minute heavily staining nuclei. The nuclei may be located, as in the chief cells, either at the inner or basal pole of the cell. Often the position of the nuclei of the mitochondria-rich cells is opposite to that occupied by the nuclei of the adjacent chief cells (Fig. 8). In the irregular cells found outside the follicular walls, the position of the nucleus is usually eccentric. The nucleoli are variable both in size and staining. Mitoses are rarely seen.

The Mitochondria of the Mitochondria-rich Cells

The *number* varies, as in the chief cells, with the state of hypertrophy and involution. In the giant cells they are increased in proportion to the increase in the size of the cell. In form they vary like those in the chief cells, being filamentous whenever the size of the cell permits and granular when the cell is flattened. It is significant, however, that the filaments are not as wavy or undulant as the filaments in the tall chief cells. They are uniformly distributed throughout the cytoplasm, but are more closely packed than in the chief cells; and there is not that tendency for dense accumulations to appear at the inner border of the tall cells, as invariably occurs in the tall chief cells. As in the chief cells, with any alteration in the position of the nucleus there is a change in the position in which the mitochondria are more numerous. A striking picture is seen when, along with the reversal of the nuclear position, the mitochondria are more numerous at the opposite poles of adjacent mitochondria-rich and chief cells (Fig. 8). This phenomenon may have some bearing on the question of the reversal of polarity in the thyroid cells.

The distinguishing characteristic between the mitochondria in these cells as compared with the mitochondria in the chief and mitochondria-poor cells pertains to the thickness or diameter of the individual elements. In all forms, whether they appear as granules, rods or filaments, their cross-sectional diameters are greater than those of the chief cells (Fig. 11). This property enables one to detect transitional cells, which are filled in part with the normal mitochondria and in part with the thicker forms. The presence of these "tran-

TABLE I
Principal Data of Cases with Relatively Few Mitochondria-rich Cells

Case no.	Age	Sex	Average pulse rate before operation	Basal metabolic rate	Clinical diagnosis	Gross and histologic findings
4748	13	F	132	+46	Hyperthyroidism	Colloid goiter, moderately involuted.
11181	17	M	80	..	Toxic adenoma	Colloid goiter, moderately involuted. No adenoma.
14777	22	F	100	+13	Goiter, adenomatous with hyperthyroidism	Colloid goiter, markedly involuted.
4763	23	F	96	..	Hyperthyroidism	Colloid goiter, markedly involuted.
11304	27	F	90	+60	Acute toxic goiter	Colloid goiter, slightly involuted.
4744	28	F	132	+140	Hyperthyroidism	Colloid goiter, moderately involuted.
11317	39	M	90	+70	Toxic goiter	Colloid goiter, moderately involuted.
4743	46	F	120	..	Hyperthyroidism	Colloid goiter, moderately involuted.
11191	48	F	90	..	Toxic goiter	Colloid goiter, moderately involuted.
11140	54	F	80	+56	Toxic thyroid	Colloid goiter, moderately involuted.

chondria-poor and mitochondria-rich cells in the different species of animals, and in the same species of animal under different conditions. When the number of mitochondria-rich cells is increased the number of mitochondria-poor cells is decreased and *vice versa*. It is noteworthy that in the markedly enlarged thyroids of dogs (from Cleveland and Detroit), the mitochondria-poor cells are increased while the mitochondria-rich cells are not more numerous than in the normal sized glands. The mitochondria-poor cells in the animal glands are found singly or in small groups in occasional follicles peripherally (like the "ovoid" cells of Bensley), in the interfollicular spaces, in the lumina of the follicles, and rarely they replace entire follicles. They participate in the "budding" processes in the animal glands just as the mitochondria-rich cells in the human gland, and are found in large numbers in the interfollicular spaces in the form of regenerating buds. The mitochondria-poor cells are almost invariably round or oval in outline; the lateral walls are not parallel but show a distinct convexity which causes an indentation of the walls of the adjacent cells. The nuclei vary in size and staining. Mitoses are occasionally seen (Fig. 9).

The Mitochondria of the Mitochondria-poor Cells

There are relatively fewer mitochondria in the mitochondria-poor cells than there are in the chief cells. They are rarely filamentous, but are rod-like in the taller, and granular in the flatter cells. Lying in the unusually clear cytoplasm they may be diffusely scattered, although they are frequently concentrated at the periphery of the cell or form a ring about the nucleus. In all forms their diameters are much smaller than those in the chief cells, indeed, the granular types are barely visible. These fine mitochondria are not as stable as those of the mitochondria-rich and chief cells, and are very easily destroyed by technical errors and chemical agents.

DIFFERENTIAL STAINING OF THE MITOCHONDRIA-RICH AND THE MITOCHONDRIA-POOR CELLS

In tissues which had been placed originally into fixing solutions containing chromates, or were chromated after having remained for periods varying from a few days to fifteen years in formalin, the mitochondria-rich and mitochondria-poor cells are distinguishable

from the chief cells by differences in the density of staining of the entire cytoplasm. This is true in sections in which the mitochondria were poorly stained, or had been stained by methods not intended to reveal the mitochondria. For example, in sections stained with hematoxylin and eosin or with iron hematoxylin, the cytoplasm of the mitochondria-rich and mitochondria-poor cells stains uniformly darker and paler respectively than the cytoplasm of the chief cells. The reasons for these differences have not been determined. The addition of osmic acid to the chrome fixatives often intensifies these differences but invariably results in less satisfactory differentiations of the mitochondria in the three types of cells. The presence of acids in the fixing solutions, such as acetic acid employed in Zenker's fluid, invariably destroys both the mitochondria and the cytoplasmic differentiations.

Although somewhat similar in their distributions, the mitochondria-rich, the "colloid" cells of Langendorff and the "ovoid" cells of Bensley are not identical. In sections prepared by the standardized technic, an occasional mitochondria-rich cell shows the characteristic outline and dark staining background of the "colloid" cell of Langendorff. By counterstaining the sections which had been fixed in Regaud's or Zenker-formol solutions, with Mallory's anilin blue Collagen stain, or with the Ehrlich-Biondi solution, it is readily seen that the "colloid" cell of Langendorff may contain either the thick mitochondria which characterize the mitochondria-rich cells or the normal types such as exist in the chief cells (Fig. 10). The mitochondria-rich cells are not distinguishable in sections stained with Mallory's phosphotungstic acid hematoxylin after fixation in Zenker-formol, and are therefore not identical with Bensley's "ovoid" cells.

As yet we have been unable to establish any differences between the mitochondria-rich, mitochondria-poor and the chief cells as regards their participation in the elaboration of the visible secretory products of the thyroid cell.

THE MITOCHONDRIA-RICH CELLS IN THE HUMAN THYROID

The significance of the variations in the numbers of the mitochondria-rich and mitochondria-poor cells in the different animals remains unknown. A valuable clue to the factors which determine the numbers of the mitochondria-rich cells was obtained in attempt-

TABLE 2
Principal Data of Cases with Large Numbers of Mitochondria-rich Cells

Case no.	Age	Sex	Average pulse rate before operation	Basal metabolic rate	Clinical diagnosis	Gross and histologic findings
11497	22	M	90	..	Adenoma thyroid	Colloid goiter, markedly involuted. (No adenoma.)
4760	24	M	150	..	Hyperthyroidism	Colloid goiter, moderately involuted.
11474	28	F	100	..	Toxic adenoma	Degenerating adenoma.
14775	29	F	100	+17	Exophthalmic goiter	Colloid goiter, markedly involuted.
11104	35	M	105	..	Basedow's disease	Colloid goiter, moderately involuted.
14776	35	F	100	+75	Graves' disease with hyperthyroidism	Colloid goiter, markedly hyperplastic.
11193	36	F	120	..	Toxic goiter	Colloid goiter, moderately involuted.
4753	36	F	130	..	Hyperthyroidism	Multiple small adenomas, hyperplastic and involuted.
11488	39	F	90	..	Toxic adenoma	Adenoma, moderately involuted.
4754	43	M	Hyperthyroidism	Multiple small adenomas, hyperplastic and involuted.
11136	43	F	80	..	Basedow's disease	Degenerating adenoma.
11131	44	F	100	..	Basedow's disease	Multiple small adenomas, hyperplastic and involuted.
11144	45	M	100	..	Basedow's disease	Multiple small adenomas, hyperplastic and involuted.
4761	45	F	68	..	Adenoma thyroid	Degenerating adenoma, markedly involuted.
11422	45	F	80	..	Colloid goiter	Multiple small adenomas, hyperplastic and involuted.
11453	47	F	105	+60	Toxic adenoma	Hyperplastic adenoma.
11274	50	M	85	..	Adenomatous thyroid	Degenerating adenoma.
11429	54	F	90	..	Toxic adenoma	Adenoma thyroid.
4759	58	F	80	..	Adenoma thyroid	Degenerating adenomas.
4762	58	F	116	..	Adenoma thyroid with hyperthyroidism	Multiple small adenomas, hyperplastic and involuted.
4752	65	F	130	+114	Hyperthyroidism	Multiple small adenomas, hyperplastic and involuted.

centage increase in the basal metabolic rates are noticeable between the cases in both groups.

It is evident that the different *clinical diagnoses* were based upon various opinions of the etiology of the associated symptoms, and hence, it was necessary to resort to a classification used by Marine in order to correlate the data. After tabulating the cases with symptoms, and the cases without symptoms under the headings of "exophthalmic goiter" and "simple goiter" respectively, as shown in

TABLE 4

Distribution of the Cases in the Two Groups according to the Anatomic Diagnoses

	Group I	Group II
Adenomatous glands.....	0	15
Non-adenomatous glands	10	6

Table 3, it is seen that in this series the presence of large numbers of mitochondria-rich cells cannot be correlated with the clinical symptoms, or with the various clinical classifications of thyroid disease. It should be noted also that although all but one of the cases of "toxic adenoma" fall into Group II, all of the adenoma cases without symptoms fall into the same group.

Anatomic diagnoses: The specimens were classified according to the gross and microscopic findings into adenomatous and non-adenomatous glands. Table 4 shows that no case showing adenomatous changes falls into Group I.

SUMMARY OF THE FINDINGS IN THE HUMAN GLANDS

In a small series of cases, the presence in the thyroid of large numbers of cells, whose increased mitochondrial contents were independent of the size of the cells, could not be correlated with the symptoms which characterize clinically distinguishable diseases of this organ. There is abundant evidence that the cells with increased mitochondrial contents are intimately associated with those changes in the gland which are associated with the age of the individual or with the formation of the so-called adenomas, or both.

ing to correlate the wide variations in the numbers of the cells with the clinical data and with the anatomic alterations in a series of thirty-one human glands. These cases were divided into two groups: Group I, included the cases whose glands contained relatively few mitochondria-rich cells or, as in the animal glands, these cells rarely filled entire follicles; Group II, included those cases whose glands

TABLE 3

Distribution of the Cases in the Two Groups according to the Clinical Diagnoses

	Group I	Group II
I. <i>Exophthalmic Goiter</i>		
"Exophthalmic goiter" or "Basedow's,"	0	5
"Hyperthyroidism"	4	4
"Graves' with hyperthyroidism"	0	1
"Toxic adenoma"	1	4
"Toxic goiter"	3	1
"Toxic thyroid"	1	0
"Adenomatous goiter with hyperthyroidism"	1	1
	—	—
Total	10	16
II. <i>Simple Goiter</i>		
"Colloid goiter"	0	1
"Adenomatous thyroid"	0	1
"Adenoma thyroid"	0	3
	—	—
Total	0	5

contained large numbers of the mitochondria-rich cells which occupied entire follicles and groups of follicles in a single section. The principle data of the cases in each group are given in Tables 1 and 2.

Age appears to be a factor in determining the relative numbers of the mitochondria-rich cells since there is a predominance of older cases in Group II. It must be borne in mind, however, that with advancing age there is a concomitant increase in the frequency and degree of anatomic alterations in the gland, and that the factor of age cannot be separated from another factor to be taken up presently, namely, the presence of adenomatous changes. Sex apparently has no bearing on the distribution of the cases in the two groups.

We are unable to correlate the diverse symptoms which are clinically attributable to disturbances of the thyroid gland except to note that the pulse rate in cases in Group II appears to be slightly higher than in those in Group I; and that no differences in the per-

duced. We believe that their findings show a direct relationship between mitochondrial alterations and histologic changes, but from the methods used, it might be inferred that either the iodine metabolism of the gland is rapidly altered or that the histologic changes were brought about by factors other than by the production of marked and rapid alterations in the iodine content of the gland.

The results obtained from experimental studies in animals and the findings in thirty-one human cases, show that a correlation cannot be established between the clinical symptoms of disturbances in the functional activity of the gland and the alterations in the number, form and distribution of the mitochondria in the thyroid cell. Moreover, assuming that Goetsch may have encountered the mitochondria-rich cells in the so-called "toxic" adenoma, the fact that these cells are as numerous in adenomas from cases without symptoms, at the time of operation, as they are in adenomas from cases presumably showing symptoms of thyroid disorders, makes it appear unlikely that the presence of cells with large amounts of mitochondria are associated with the clinical evidences of thyroid disorders. It is possible, however, that with a larger series and with more carefully studied clinical histories some relationship may be found to exist between one or more of the diverse manifestations of thyroid disease and the presence of these mitochondria-rich cells.

An important finding recorded in this communication, we believe, is the definite association of large numbers of mitochondria-rich cells with the presence of adenomatous changes in the human gland. In the simple diffuse hyperplasias in the animal glands the numbers of the mitochondria-poor cells were increased. The fact that the mitochondria-rich cells normally are present in larger numbers in the human gland, in which adenomas occur far more often than in those of all other animals, suggests that in the adenomas, the presence of cells with large amounts of mitochondria is not accidental. Future studies may disclose the significance of this association.

From the fact that constant alterations in the number, form and distribution of the mitochondria of the thyroid cell accompany the histologic changes which Marine showed were dependent upon variations in the percentage-iodine content of the gland, it might be inferred that these alterations were directly concerned with the iodine metabolism of the cells. Similarly, from the fact that the adenomas, which contain enormous numbers of mitochondria-rich cells, show

DISCUSSION

Descriptions of cells resembling those we have designated as the mitochondria-rich and mitochondria-poor, as contrasted with the chief cells, are occasionally met with in papers dealing with the cytology of the thyroid gland in relation to its secretory processes. As far as we have been able to ascertain from the abundant literature dealing with the rôle of mitochondria in the secretory or any other specific activity of a highly specialized cell, and from our own studies along these lines in tissues other than the thyroid, it appears very unlikely that the mitochondria are directly associated with the formation of the visible secretory products of the cell, or with any specific functional activity of any specialized cell. Indeed, in those cells which elaborate visible secretory products, it seems that the alterations of the mitochondria are entirely passive; that during the elaboration or formation, storage and discharge of these products, the alterations of the mitochondria result from as yet entirely unknown factors in addition to the altered volumetric conditions existing within the cells during these secretory phases. As regards their relation to the colloid in the thyroid gland, the observations here recorded show that the cells with larger or smaller amounts of mitochondria do not differ from the chief cells since the amounts of colloid within the follicle vary inversely with the degree of hypertrophy of the respective cells. A review of the literature dealing with the relationship between mitochondrial alterations and secretory processes of the thyroid cell, along with additional observations which confirm these deductions, have been recently contributed by Ma⁶ and Key⁷.

Cramer and Ludford⁸ concluded from the observation that mitochondrial alterations occur in the thyroid gland following the exposure of the animal to environments of high or low temperatures and following the subcutaneous injection of B-Tetrahydronaphthylamine, that a very clear relationship may be demonstrated between the functional state of the gland and the mitochondria. The variations in the functional activity of the gland, ranging from "intense activity" to "complete inactivity," were estimated by histologic standards based upon their findings in previous studies when, by using the same experimental procedures, it was assumed that variations in the functional activity of the thyroid had been pro-

in the different animals and in the same animal under different conditions, especially with the age of the animal, and that transition forms are present, suggest that these cells may be regarded as different types of cells in the sense that the differently staining cells in such organs as the anterior lobe of the pituitary, the parathyroid and the islands of Langerhans are so designated. That they are not indicative of pathologic states of the thyroid cell, as opposed to the "normal" chief cell is the fact that they respond to iodine variations. In the other organs mentioned, the different types of cells are distinguishable by variations in their mitochondrial contents as well as by the other cytologic criteria. Homans ¹⁰ has shown that in diabetes it is chiefly the B cells (Bensley) ¹¹ of the islands of Langerhans that are affected. Along similar lines it may be possible to correlate the three types of cells with specific functional disturbances of the thyroid, and upon the assumption that the mitochondrial alterations represent variations in the basic activities, it may lead to a better understanding of the relationship between the functional and morphologic alterations in the thyroid gland.

SUMMARY AND CONCLUSIONS

In the human thyroid, as in the glands of all animals examined, the definite and constant alterations of the mitochondria occurring during hypertrophy and involution of the cells in the adenomatous formations are similar to those which occur in the normal or non-adenomatous thyroid tissue. These alterations cannot be associated with any specific activity of the thyroid cells, nor can they be correlated with the known clinical syndromes of thyroid disease.

In the thyroid gland of all the animals examined, certain cells are found which contain larger or smaller amounts of mitochondrial substance than are contained in the predominating or chief cells. The variations in the amounts of the mitochondria in these cells are independent of the variations in the size of the cells. These mitochondria-rich and mitochondria-poor cells undergo hypertrophy and involution like the chief cells. The numbers of the mitochondria-rich and mitochondria-poor cells vary in different species of animals and in the same species under different conditions.

The mitochondria-rich cells are more numerous in the human gland than in the glands of any of the common laboratory animals.

a lag in the histologic response to iodine administration (Marine), it might be inferred that these cells are associated with the altered iodine metabolism of the adenomas. In this way, a close relationship between a specific activity and the mitochondrial content of highly specialized cells appears to be established. However, the differences between the mitochondria in the mitochondria-rich and chief cells may not be dependent upon differences in the iodine metabolism of these cells for the following reasons: (1) During the wide range of variations in the iodine metabolism occurring while the hypertrophic and involuted states are being produced experimentally, the mitochondria in the chief cells showed no tendency to become thicker or thinner. (2) Cells in part filled with the thick mitochondria of the mitochondria-rich cells and in part with the normal mitochondria would have to be interpreted as showing different stages of iodine metabolism existing within the same cell. (3) The thick mitochondria of the mitochondria-rich cells and the thin mitochondria of the mitochondria-poor cells undergo the same alterations during hypertrophy and iodine involution as the "normal" mitochondria of the chief cells.

On the basis that the mere presence of the mitochondria must exercise some influence on one or more of the cellular activities, or that they result from some form of activity common to all living cells, it is generally accepted that they are associated with the basic or the so-called vegetative activities of cells such as respiration and nutrition. From this point of view, the mitochondria-rich, mitochondria-poor and chief cells represent different states of vegetative activities of thyroid cells. How their specific functions are affected by variations in their basic activities remains to be determined. As was pointed out previously, it seems that the thyroid gland whose anatomic and physiologic manifestations are intimately bound up with its iodine metabolism, is especially suited for experimental investigations pertaining to the possible rôle of mitochondria in cellular activities, since variations in its iodine metabolism may be experimentally controlled. In a future paper it is hoped that further data along these lines will be presented.

The significance of mitochondrial alterations, from the standpoint of the functional activities of the cells containing them is unknown. However, the facts that the mitochondria-rich and mitochondria-poor cells are present in normal thyroids, that they vary in number

DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Filamentous mitochondria in the tall cells of the rat thyroid. $\times 3000$.

FIG. 2. Rod-like and granular mitochondria in the flattened cells of the rat thyroid. $\times 3000$.

PLATE 109

FIG. 3. Follicle of mitochondria-rich cells in the rabbit thyroid. $\times 900$.

FIG. 4. Mitochondria-rich cells in the human thyroid. $\times 75$.

PLATE 110

FIG. 5. Mitochondria-rich cells lying within the lumina of the follicles in the human thyroid. $\times 400$.

FIG. 6. Follicles of mitochondria-rich cells at the periphery of a degenerating adenoma in the human thyroid. $\times 150$.

PLATE 111

FIG. 7. Follicles of mitochondria-rich cells near the center of a degenerating adenoma in the human thyroid. $\times 150$.

FIG. 8. Mitochondria-rich cells in a papillary bud in the human thyroid. $\times 250$.

PLATE 112

FIG. 9. Mitochondria-poor cells in the guinea-pig thyroid. $\times 350$.

FIG. 10. Schematic drawing of hypertrophic and involuted thyroid follicles showing at (a) chief cells, at (b) mitochondria-rich cells, at (c) mitochondria-poor cells and at (d) Langendorff cells.

In the human gland the variations in the number of the mitochondria-rich cells are associated with such factors as age, the presence of adenomatous changes in the gland, or both. In a small series of cases the presence of large numbers of mitochondria-rich cells could not be correlated with the associated clinical symptoms of thyroid disease, nor were they more numerous in the adenomas from cases with, than from cases without associated symptoms of thyroid disease. The significance of these findings from the standpoint of the relationship between mitochondrial alterations and variations in the functional activity of the thyroid gland remains to be determined.

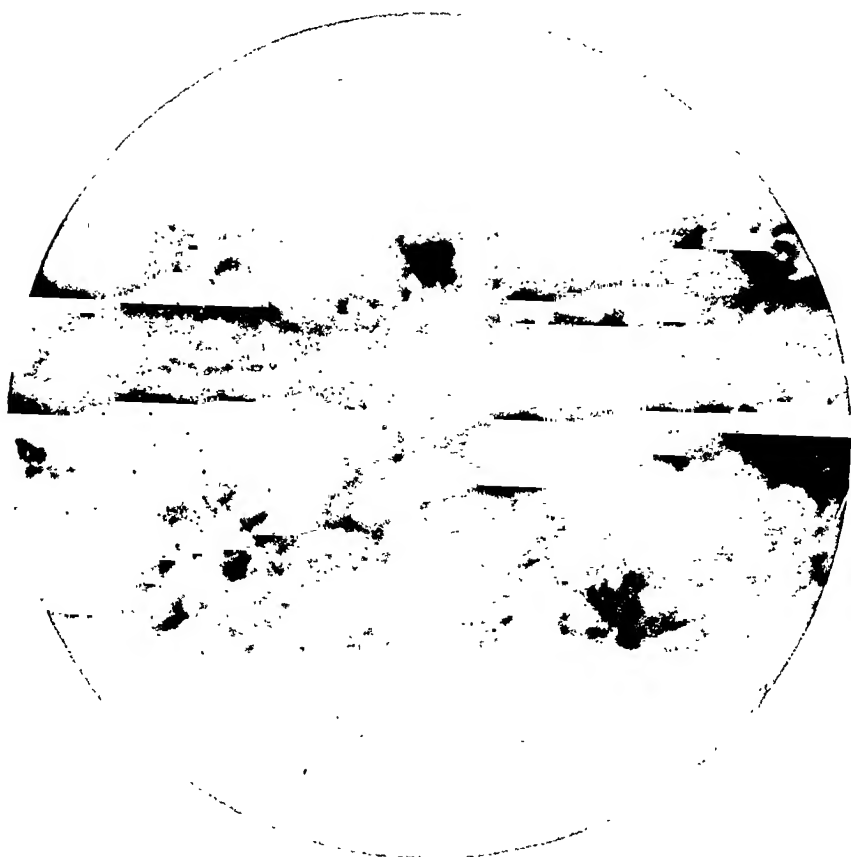
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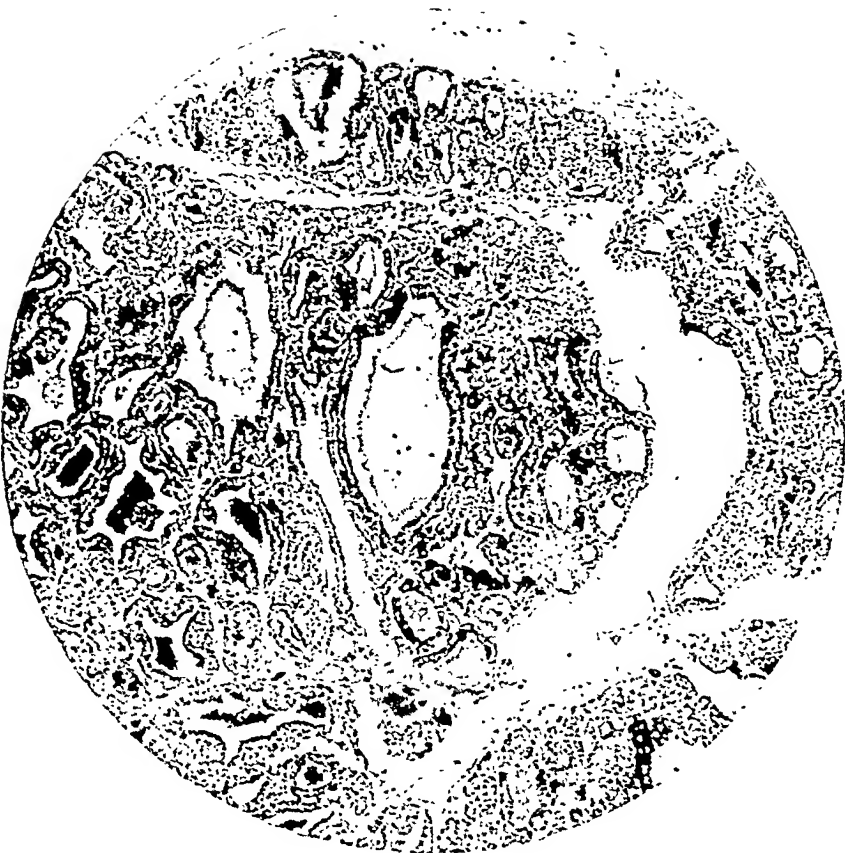
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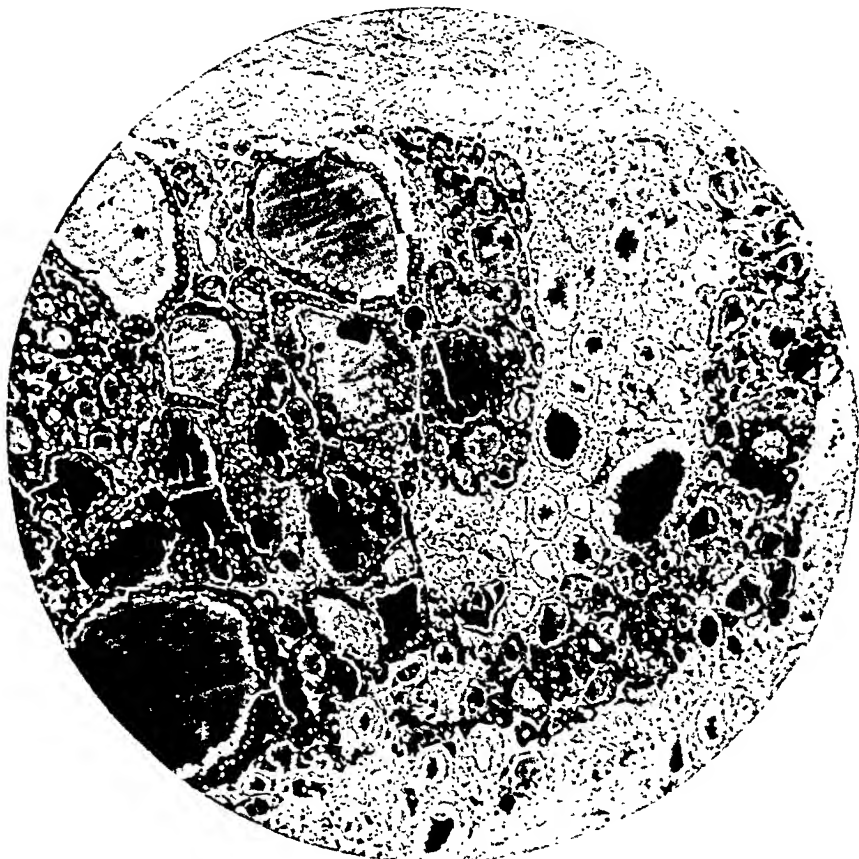
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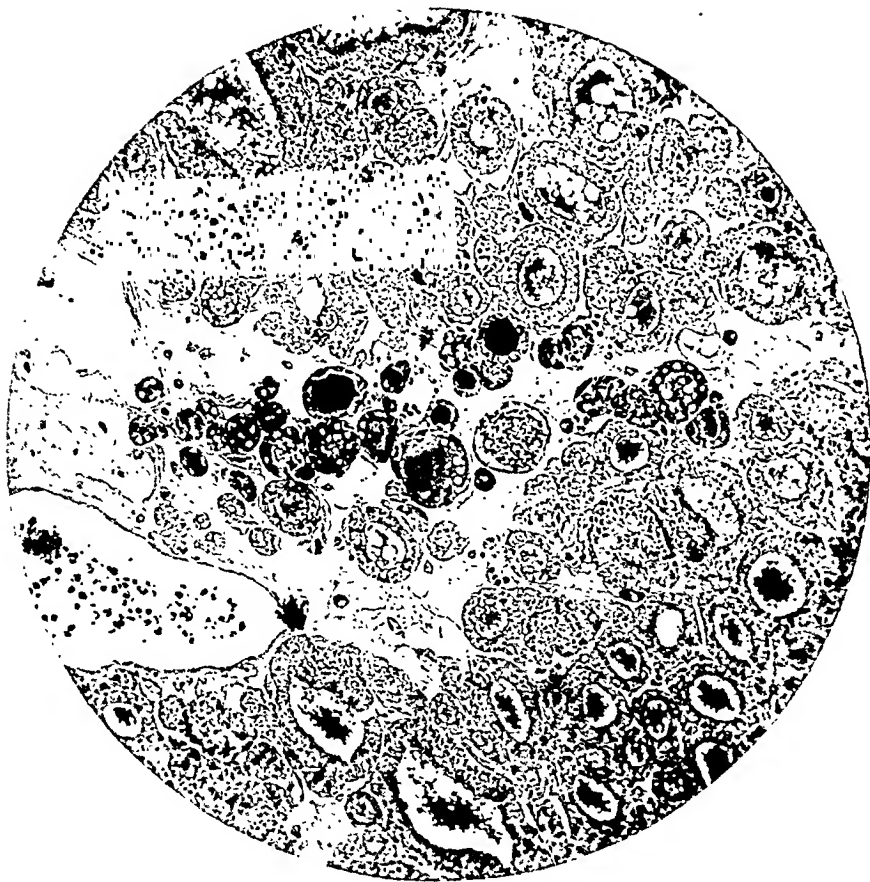
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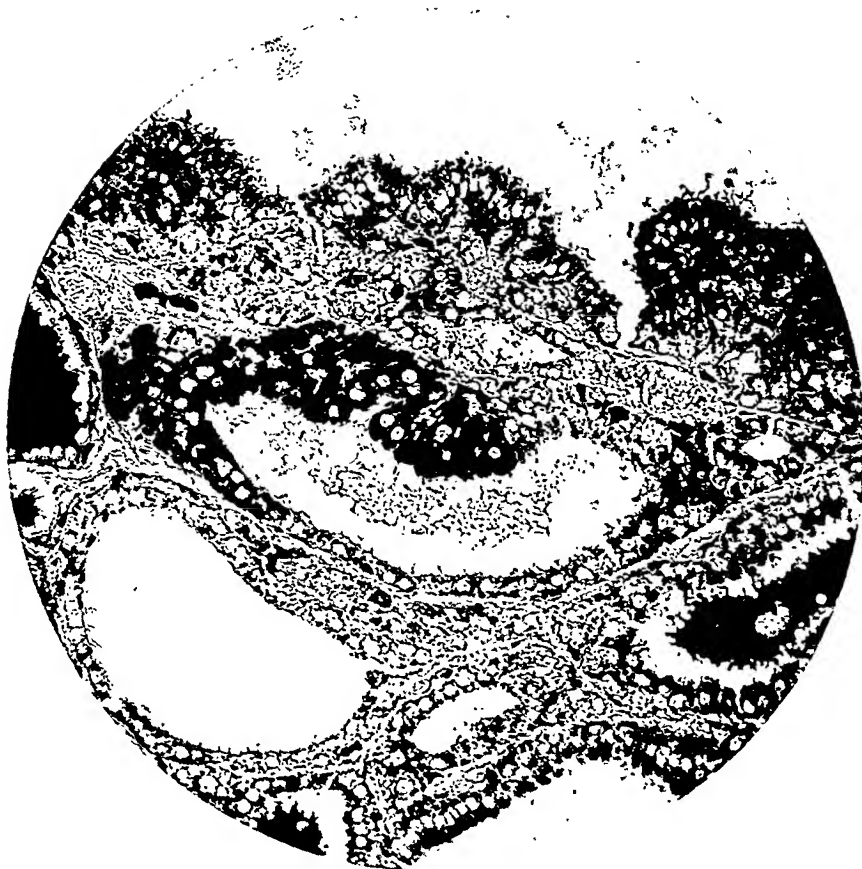
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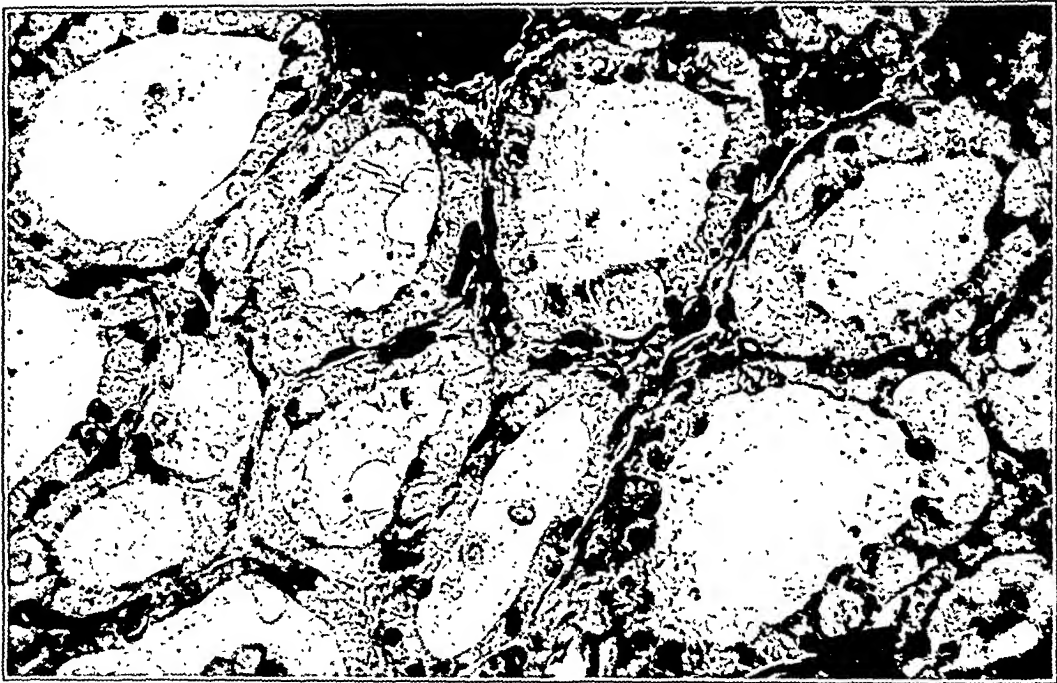
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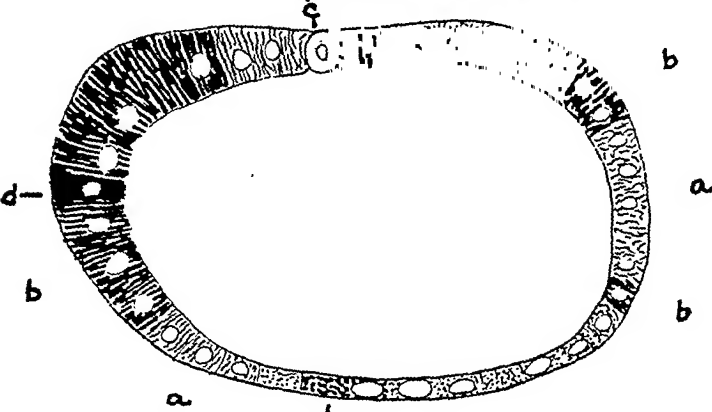
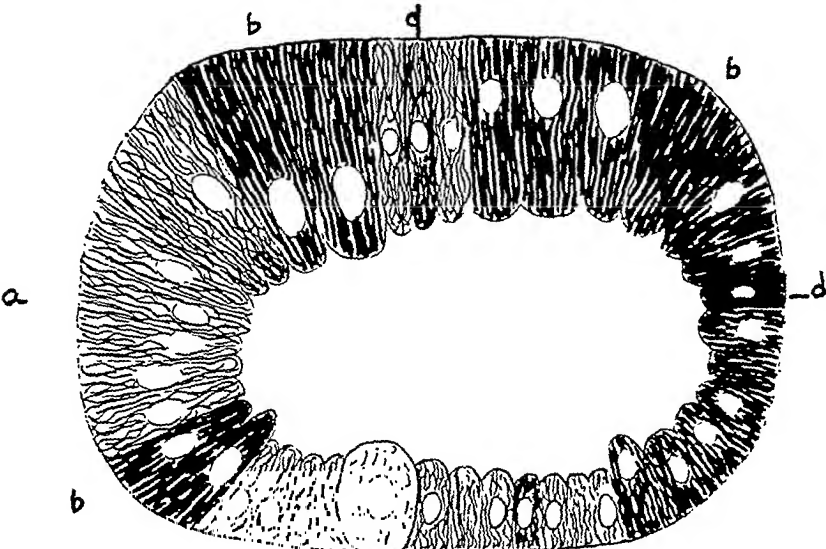
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Seecof

Studies on Mitochondria



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Vidal, Haab and others. Juliusberg¹ in 1905 showed that the virus could pass through a Chamberlin filter. The filtrate inoculated into three persons was negative in two, but induced the disease typically in one after an incubation of fifty days. More recently Wile and Kingery² have shown the filterability of the virus through the finest Berkefeld filters.

REVIEW OF FORMER STUDIES

Several reviews of the earlier and more recent literature are available and no attempt will be made to cover the earlier work exhaustively.³ In 1891 Henderson and Patterson described rather large oval hyaline structures in the superficial layers of the epithelial nodules which were regarded as specific parasites, and these well defined structures have since been called molluscum bodies. The most important morphologic investigations of molluscum contagiosum have been those of Lipschütz,⁴ who observed in fresh preparations from the epithelial nodules innumerable, non-motile, spherical bodies, uniform in size and measuring in stained preparations 0.25 micron in diameter. In smears these minute structures were stainable by Loeffler's flagella stain, and appeared as discrete, round, uniformly colored bodies, quite characteristic and easily distinguished from any other stainable material in the preparation. In sections he succeeded regularly in demonstrating within certain swollen epithelial cells of the cutaneous nodule similar bodies in enormous numbers, closely packed together but sufficiently discrete to be resolved with high magnification. These elementary bodies, Lipschütz regarded as the etiologic agent of the disease, and has named them *Strongyloplasma hominis*. The discovery of the elementary bodies was confirmed by von Prowazek.⁵

In stained sections Lipschütz recognized coarser and more irregular bodies, within the cytoplasm of the affected cells, which stained like nucleoli and were regarded by him as nuclear derivatives expelled into the cytoplasm under the influence of the infection. These were identified as the same bodies earlier described by Kuznitsky and by MacCallum. The nuclear particles, he believed, were in every way distinct from the elementary bodies, differing from them in size, staining qualities and number. He recognized no genetic relation between them.

In a more recent cytologic investigation of molluscum contagio-

A CYTOLOGIC STUDY OF MOLLUSCUM CONTAGIOSUM *

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INTRODUCTION

In the present limited extent of our knowledge of the virus diseases and the lesions associated with them, it is of importance to analyze with the greatest care the cellular changes which an infection by each virus induces; and especially those changes associated with "inclusion bodies," whether intranuclear, intracytoplasmic or both, that seem to be specific for a certain virus or for a group of viruses. It is of particular moment to arrive at a correct interpretation of such inclusions as those associated with molluscum contagiosum which appear to be in part at least composed of myriads of minute bodies. In size, these seemingly are such as to satisfy the physical requirements which would permit them to pass through the pores of a porcelain filter. Their number, morphology and staining reactions suggest that they are microorganisms and the etiologic agent of the disease. A sound morphology is an important basis for cultural investigations, the successful accomplishment of which would be necessary for the permanent establishment of an etiologic agency.

CLINICAL MANIFESTATIONS

Molluscum contagiosum is an infectious disease of the human skin which is characterized by the formation of multiple, discrete, cutaneous epithelial nodules averaging about 2 mm. in diameter. There are numerous thorough clinical accounts of the disease and of the histology of the lesions. It is more commonly an infection of children, and it has frequently been observed that the disease has spread in a short time from one child to many others in close association, as in schools.

Experimental transmission of molluscum contagiosum was accomplished for the first time by Retzius in 1871. This was confirmed by

* Received for publication April 28, 1927.

most useful fixative in our hands has been Regaud's fluid. The tissue was fixed in an ample amount of this fluid for twenty-four hours, then transferred for three days to 2.5 per cent potassium bichromate. For the demonstration of mitochondria, paraffin sections were stained by the acid fuchsin-methyl green method.⁷ A variation in this technic which we have not seen elsewhere recorded, consisted in mordanting the sections (before staining in the anilin acid fuchsin) for one minute in a 1 per cent solution of potassium permanganate, washing thoroughly in water and staining directly in the usual way without treatment with oxalic acid. A very sharp staining of mitochondria has been obtained in this manner. Another useful method has been Mallory's phosphotungstic acid hematoxylin, applied without previously treating the section with potassium permanganate and oxalic acid. This method stains the intracellular masses of elementary bodies a deep blue and almost specifically, but the definition of the individual elements is not so sharp as in the acid fuchsin preparations. For demonstrating keratohyalin and the blue cytoplasmic network between the masses of elementary bodies, the carbol-anilin-fuchsin stain,⁸ applied for one minute and counterstained to the intensity desired with Löffler's methylene blue, has been of considerable service.

PATHOLOGY OF THE LESION

A typical well developed lesion has the gross appearance of a shot-like papule in the epidermis measuring about 2 mm. in diameter, with a minute round orifice at its apex through which one sees a pearly white central core which is firm. The tiny nodule is formed by a circumscribed overgrowth of squamous epithelium which forms a rounded, often somewhat lobulated, tumor-like mass which elevates the surrounding uninvolved epidermis. There is little or no inflammatory reaction about the nodule in the corium. In the germinal layer of epithelium one may see fairly numerous cells in mitosis; and the germinal cells of the lesion are considerably larger than corresponding cells of the normal skin. The cytoplasm is pale and contains numerous mitochondria, appearing as tiny granules or filaments uniformly distributed. The nucleus is correspondingly large and contains one or more rather faintly staining nucleoli. There are no abnormalities in these cells except their relatively large size. The epithelium of the nodule shows much the same stratification as that

sum, Sanfelice⁶ observed that the most significant changes in the cell take place in the nucleus. Both the nucleus and cytoplasm of the cells of the Malpighian layer in the affected area enlarge. The nucleolus also increases in size and stains a deeper red with Mann's stain than nucleoli of normal cells. In addition to the red-staining nucleolus, he observed sometimes two small intranuclear bodies staining blue. The red-staining nucleolus was observed to become extruded from the nucleus so that it came to lie entirely in the cytoplasm. According to this author, the extruded nucleolus, after a while, increases in size and changes its staining reaction, retaining the methyl blue instead of the eosin. When stained blue it is always surrounded by a clear areola which gradually shades into the cytoplasm and, when it occupies a large part of the cytoplasm, it has a granular appearance and vacuoles can be distinguished in it. The inclusion by enlarging takes the form of a typical molluscum body. It then has a finely granular appearance and stains red. Sanfelice concludes from these observations that molluscum bodies have their origin from nucleoli which, under the influence of the infection, become extruded into the cytoplasm. Lipschütz, while admitting a nuclear origin for certain cytoplasmic granules in the affected epithelial cells, recognized no genetic relation between them and the elementary bodies which constitute the main bulk of the molluscum bodies.

THE PRESENT PROBLEM

The present cytologic investigation of molluscum contagiosum was undertaken to determine whether, in our opinion, the elementary bodies of Lipschütz in the lesions of molluscum contagiosum would be acceptable with the evidence available as possible microorganisms, or whether they should be more properly classed as products of cytoplasmic or nuclear degeneration. It was of interest in the investigation also to study their relation to certain cytoplasmic constituents especially mitochondria, since technical methods for the demonstration of these normal cytoplasmic constituents had not been employed in previous studies.

TECHNIC

Several epithelial nodules 1 to 2 mm. in diameter, fixed immediately after excision in a number of fluids, have been examined. The

condensed in the form of an irregular network between the vacuoles marking the cell off into compartments, and the vacuoles become uniformly filled with minute, fairly discrete, bodies which stain pink with acid fuchsin. These minute structures are the elementary bodies of Lipschütz. Every epithelial cell in the lesion is not thus affected. Some undergo an apparently normal differentiation with the formation of masses of keratohyalin, and these become more or less compressed between the larger cells. The finely granular intracellular compartments continue to increase in size until they fill the greatly enlarged cell except for the narrow cytoplasmic network between them, and in this fully developed form, the entire mass of granules and basophilic cytoplasm seem to separate from a peripheral cellular membrane, and as the surface is approached and keratinization of unaffected cells has proceeded to completion, the intracellular mass of granules and cytoplasm shrink from the membrane and fuse to become an oval hyaline body lying within a framework composed of its own membrane and the remnant of surrounding keratinized cells. The main factor in the hyalinization of the intracellular mass is desiccation. The hyaline oval masses formed by a coalescence of granules and cytoplasm constitute the mature "molluscum bodies" of Henderson and Patterson. They are not formed by a sort of keratinization as suggested by Lipschütz, but by fusion and desiccation of the elementary bodies and the intervening cytoplasm.

It is evident from our preparations that the particles which appear to be extruded from the nucleus have no part in the composition of the elementary bodies, but dissolve and become a part of the basophilic cytoplasm which has a central position within the cell. It is within this altered cytoplasm that the vacuoles develop, about which and eventually within which tiny bodies occur having no counterpart among the cellular constituents. Mitochondria play no part in the formation of the elementary bodies. In fact the elementary bodies constitute, from all cytologic appearances, a new substance which increases enormously in bulk in the affected cells, and not by accretions to the size of individual bodies, but by a proliferation of innumerable bodies of uniform size, form, and staining qualities. The cytologic changes are in every way consistent with an active growth of a very minute living microorganism. Smear preparations, made by stroking a glass slide over the pearly core of a lesion mois-

of normal skin, and the first significant changes occur just peripheral to the germinal layer. The cells and nuclei enlarge. The nucleoli become more prominent and more deeply stained, often presenting a vacuolated structure. It is in such cells that one may observe what appears to be an extrusion of nucleoli into the cytoplasm. The mitochondria in these cells are granular and tend to be grouped near the nucleus. Within the cytoplasm there may be one or several round bodies which have the size, shape and staining reaction of nucleoli. As was observed by Sanfelice, nuclei may be found which show nucleoli in the process of extrusion. These intracytoplasmic bodies often lie among a dense group of granular mitochondria. The mitochondria in this layer are coarser and more easily and intensely stained than those in the germinal layer. In certain cells the bodies which have the appearance of extruded nucleoli possess a sharp contour, in other cells they seem to be fading away as if dissolving in the cytoplasm and becoming basophilic in reaction. Again they may be surrounded by a definite and broad zone of material which stains bluish with Mallory's phosphotungstic hematoxylin and pinkish with fuchsin. This zone has the appearance of a halo in the cytoplasm. At about this stage in the development of the cell the cytoplasm in its center becomes more basophilic, and sometimes the nucleolus-like particles are arranged more or less regularly about the periphery of this basophilic portion. The particles gradually disappear and it is a question whether their presence does not add something which aids or causes the basophilic differentiation of the central mass of cytoplasm.

Also about the periphery of the basophilic cytoplasmic area there appear numerous small vacuoles which have a somewhat irregular outline owing to the fact that a new and apparently granular substance, becomes arranged about them. The mitochondria appear as coarser granules, less numerous and more prominent in the neighborhood of the nucleus. The material about the vacuoles takes a bluish stain in phosphotungstic acid hematoxylin preparations and pinkish with acid fuchsin, and is identical in this respect with the more abundant finely granular material which constitutes the bulk of the cells in later stages. The cell enlarges by an increase in number and size of the vacuoles; the nucleus becomes pushed to one side and is flattened or crescentic in shape. Several coarsely granular mitochondria are situated near it. The basophilic cytoplasm becomes

nucleoli seem to be extruded into the cytoplasm, there is no indication that they play any morphologic part in the formation of the minute elements. The nucleoli disintegrate and probably become a constituent of the cytoplasm which is rendered thereby more basophilic. Perhaps the addition of nuclear material enriches the cytoplasm for the growth of a virus represented by the minute bodies. Mitochondria do not participate in the formation of the elementary bodies, but remain in a distinct though modified form until the cell becomes desiccated.

No cytoplasmic element seems to be transformed into the small bodies under consideration. The latter rather tend to grow within cytoplasmic spaces, increasing rapidly in number and in total volume, possibly by direct division, as a microörganism might do.

No disintegration of affected cells is noted, from which it is inferred that the presence of the intracytoplasmic structures is comparatively innocuous. The preservation of mitochondria, in cells which show an enormous development of the minute cytoplasmic bodies, is against an assumption that the presence of the latter is due to a degeneration of the affected cell. There is little or no reaction in the corium about the lesion, which may be interpreted as meaning that the virus remains localized within the epithelial cells. The absence of a general immunity points to the same conclusion.

The morphologic evidence at hand is in favor of the view that in *molluscum contagiosum* we are dealing with an intracellular infection limited to epithelial cells, and that the minute bodies which accumulate in myriads within these cells may be the virus. Proof of such an assumption awaits experimental demonstration.

SUMMARY

1. The observation by Lipschütz that epithelial cells in the lesions of *molluscum contagiosum* contain myriads of minute bodies morphologically consistent with a filter-passing microörganism has been confirmed.

2. These bodies are not derived from extruded nucleoli, nor from any formed cytoplasmic constituent.

3. Mitochondria are neither involved in their formation nor destroyed by the changes in the cell associated with the presence of the minute bodies.

tened with physiologic salt solution, and suitably stained, confirm the cytologic appearances of an intracellular microörganism.

The smears are most satisfactorily stained in our experience by drying them in the air, fixing for one minute in absolute alcohol, washing in water and mordanting for one minute in 1 per cent potassium permanganate. After washing off the permanganate thoroughly they are stained with a few drops of carbol-anilin-fuchsin for one minute, washed and blotted. In such preparations one sees with an oil immersion objective, myriads of minute, round or slightly elliptical, discrete, uniform bodies stained a deep pink. In thicker portions of the smear they are so closely packed together as to be individually indistinguishable, but in thinner areas they are very clearly seen. Often they occur in biscuit and paired forms as if dividing. One readily gains the impression from a study of such a preparation that they are a species of microörganism; and they are identical with the elementary bodies of the intracellular vacuoles. They are not discernible in smears stained with the usual routine bacteriologic stains. Lipschütz demonstrated them successfully with Löffler's flagella stain, but owing to the precipitate formed by this method, the stain recommended above has been preferable in our own experience.

We have used various liquid and solid media in attempts to cultivate the bodies, but without success. The minute bodies have been observed to remain stainable for several weeks when suspended in physiologic salt solution and kept at room temperature.

DISCUSSION

The bodies appear first about cytoplasmic vacuoles, suggesting that the cell may react early to the presence of these minute structures in a manner analogous to macrophages which have taken up a foreign material like trypan blue, forming vacuoles about which the dye becomes concentrated in crystalline particles, constituting the so-called segregation apparatus of Evans and Scott.⁹ The affected epithelial cells of molluscum contagiosum increase greatly in volume through the increase in numbers of minute formed elements within definite compartments in the cytoplasm.

These minute elements do not appear to arise through degeneration or disintegration of any known constituent of the cell. Although

- FIG. 6. Cells showing cytoplasmic alteration. Central basophilic area surrounded by vacuoles with condensations about them. Mitochondria are granular and large.
- FIG. 7. Cell showing cytoplasmic compartments filled with elementary bodies. Mitochondria are still present in granular form about the compressed nucleus.
- FIG. 8. Two cells showing elementary bodies in cytoplasmic compartments. Three compressed cells about them unaffected by the virus, containing keratohyaline particles (black). This is the stage illustrated in Plate I, Fig. 2, before desiccation of the affected cells to form molluscum bodies.

4. The minute bodies develop about and later within cytoplasmic vacuoles which may be regarded as the cellular response to the presence of a living foreign body.

5. The view is expressed that these elementary bodies of Lipschütz may be the virus and the etiologic agent of molluscum contagiosum.

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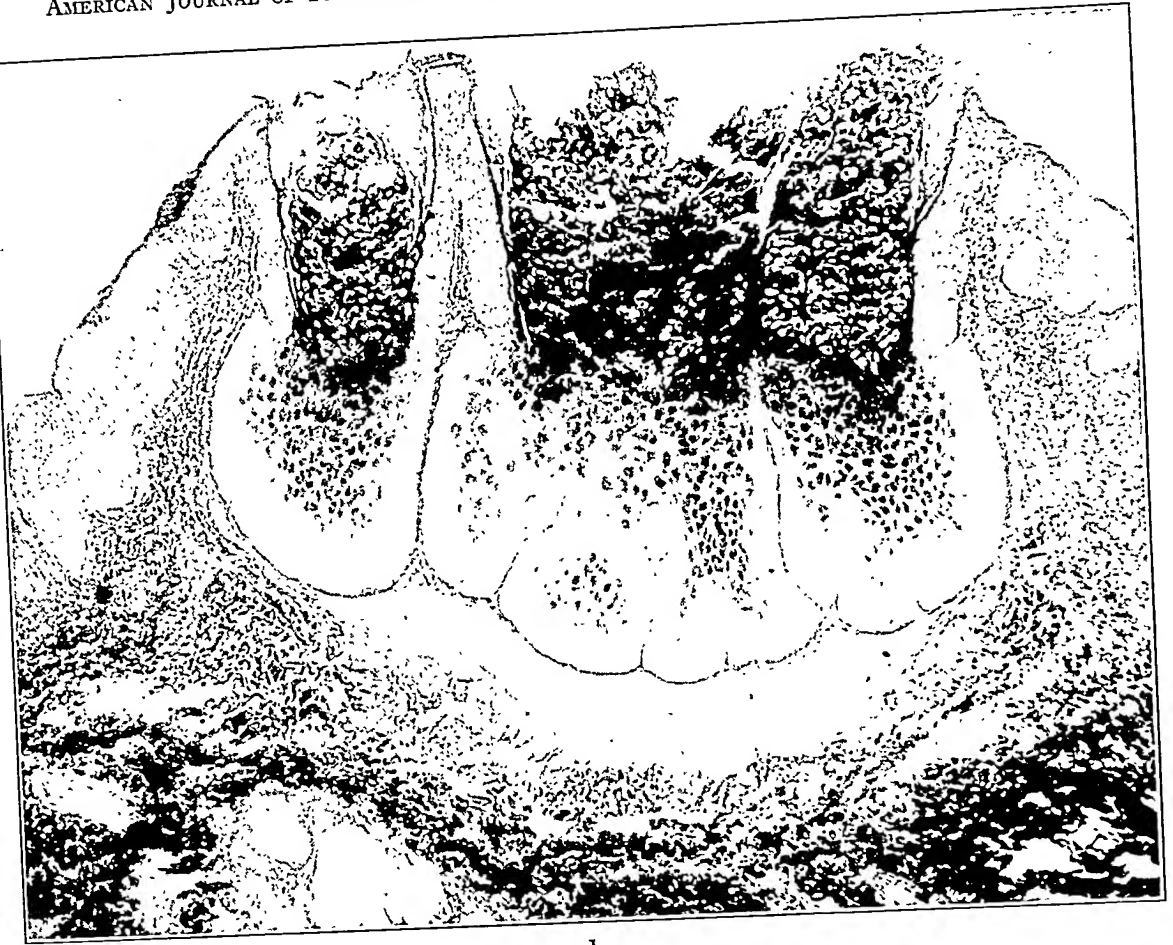
DESCRIPTION OF PLATES

PLATE 113

- FIG. 1. Lesion of molluscum contagiosum fixed in Regaud's fluid and stained with phosphotungstic acid hematoxylin. The dark areas in the epithelial cells are masses of the elementary bodies. In the desquamating portion the molluscum bodies are not stained.
- FIG. 2. High power of the same preparation to show the transformation of the intracellular masses, divided into compartments in the upper part of the picture, through a dense staining stage into typical hyaline unstained molluscum bodies.
- FIG. 3. Smear directly from lesion showing elementary bodies or *Strongyloplasma hominis* of Lipschütz, stained with carbol-anilin-fuchsin after mordanting with potassium permanganate. $\times 2000$.

PLATE 114

- FIG. 4. Cells from the basal layer of the molluscum lesion showing enlargement, numerous mitochondria and prominent nucleoli. No virus bodies present.
- FIG. 5. Epithelial cell showing extrusion of nucleoli; some of the nuclear particles being surrounded by a halo. Numerous mitochondria.

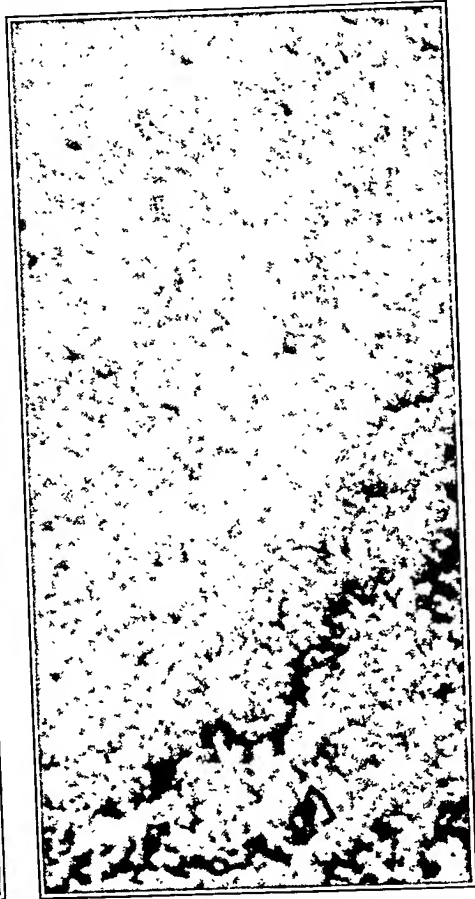


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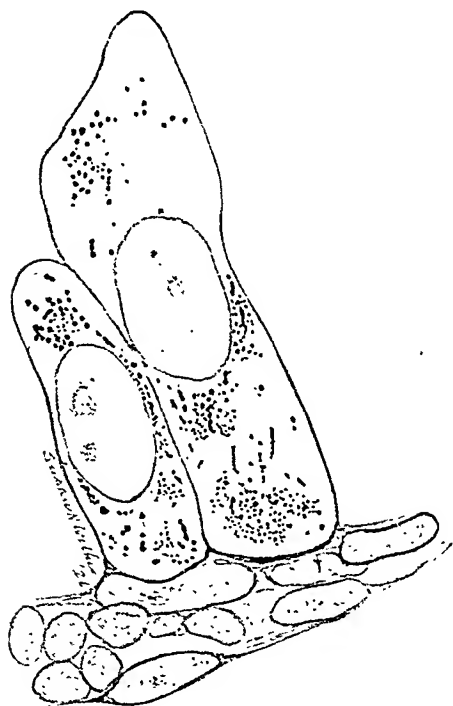
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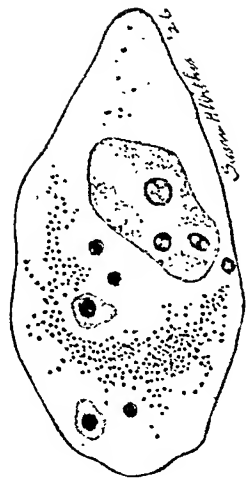


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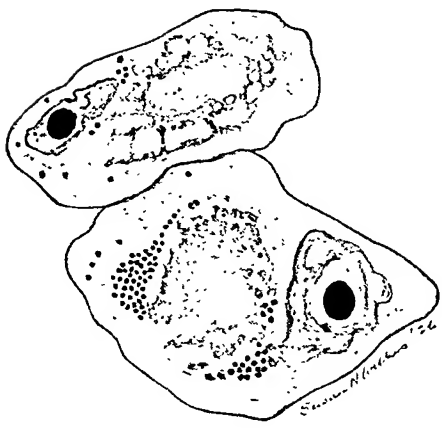
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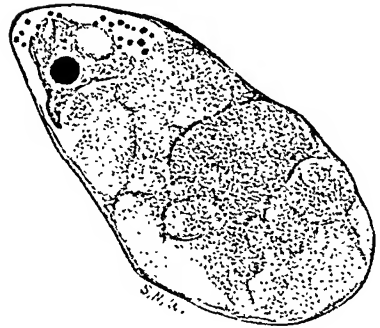
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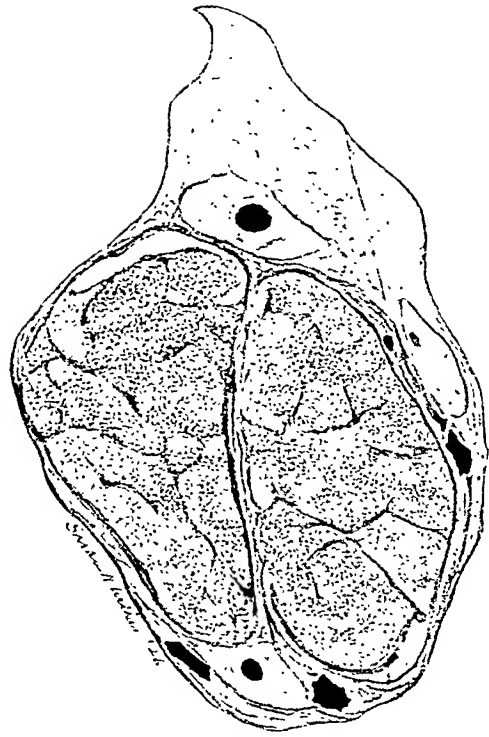
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plasm, and this, although negative evidence, indicates that they may be at least in part composed of material foreign to the cell.

In some recent studies of experimental herpetic encephalitis of rabbits the writer has observed structures within the nuclei of ganglion cells in the central nervous system, in association with changes characteristic for this virus, which it is believed have not been described before. These inclusions appear within the nuclei of altered cells in the herpetic lesion, in association with, but quite distinct from the specific herpetic bodies. They occur so far as has been observed only within nuclei of ganglion cells and are not anteceded, as well as has been ascertained, by any formed element of the cell, but appear to arise from an abnormal metabolism, or to be liberated by a disintegration of some material peculiar to nuclei of nervous tissue. They are possibly lipoidal in nature.

The specific intranuclear inclusions of the lesions of herpes simplex, first recognized by Lipschütz,¹ have been repeatedly described.⁸ They are composed of an eosinophilic granular compact mass which is distinguishable from the nucleoli and from chromatin, often lying as a distinct mass separated from the nuclear membrane by a clear zone which is unstained in the usual fixed preparations. They can be seen in fresh untreated preparations. These inclusions occur typically in epithelial cells of the spontaneous herpetic lesions of man, and are constantly present in early lesions experimentally induced in animals in whatever tissue the virus has caused a local infection. They are probably associated with the presence of herpetic virus.² In herpetic lesions of the central nervous system they are found in characteristic fashion in all types of nervous tissue, including ganglion cells, neuroglia and ependyma.

In early lesions of herpetic encephalitis the specific herpetic intranuclear bodies in ganglion cells may be found surrounded by a rather wide clear zone which in hematoxylin and eosin preparations may show no structural differentiation. If, however, the tissue be fixed immediately after death in Zenker's solution, preferably by injecting this fluid directly into the carotid arteries, and the paraffin sections stained properly with acid fuchsin-methylene blue or carbol-anilin fuchsin-methylene blue,⁹ definite morphologic structures may be demonstrated in this zone in association with and often surrounding the specific herpetic body. In suitably differentiated preparations stained by either method the bodies in question stand

NUCLEAR CHANGES OF GANGLION CELLS IN EXPERIMENTAL HERPETIC ENCEPHALITIS *

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It is well known that many virus diseases are associated with peculiar changes which take place within certain cells of the lesions; and these changes are considered to be characteristic of the virus concerned. Some viruses induce their characteristic changes within the cytoplasm alone, as in mollusum contagiosum, fowl-pox, trachoma and rabies. Others, variola and vaccinia, involve both cytoplasm and nucleus; and a third group, to which belong varicella, herpes zoster, herpes simplex, Virus III of rabbits, a disease of the salivary glands of guinea-pigs, polyhedral disease of caterpillars, Borna's disease of horses, and possibly verruca vulgaris, alter specifically the nucleus. The virus probably in each of these various diseases is intimately connected with the cells which it thus affects and it possibly multiplies within those which it specifically alters.

Certain investigators have attached a great deal of significance to the specific inclusion bodies of herpes, claiming that they are masses of virus growing within the cell, or specific products of the cell which thus reacts to the presence of the virus. Others² see in the inclusions only products of cellular degeneration or disintegration arising from changes in preformed elements of the nucleus or of the cytoplasm. However, in spite of assiduous attempts no one has as yet succeeded in inducing any of these characteristic changes in cells exclusive of the local presence of a virus.^{3, 4} Consequently, the presence of certain kinds of inclusion bodies, particularly within the nucleus, is coming to be regarded as a criterion of the presence of a virus in a lesion of unknown etiology.^{5, 6, 7}

It is characteristic of many inclusion bodies that they have the appearance of being a new substance originating within the cell. Their presence is not yet satisfactorily proved to be due to observable alterations in recognizable constituents of either nucleus or cyto-

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The rabbit is killed with ether. Twenty cc. of Zenker's solution injected from a syringe into each carotid artery. The brain is removed and fixed for twenty-four hours in Zenker's solution. Blocks through the entire brain, to include Ammon's horns, are cut for paraffin sections. Microscopically, there is advanced herpetic encephalitis at the base including Ammon's horns and cerebrum laterally. Numerous ganglion cells show typical herpetic inclusions and of these, many, especially in Ammon's horn, contain in addition spherical bodies in the clear zone about the herpetic inclusions.

The fact that the intranuclear bodies which are described above have been demonstrated only within ganglion cells of the central nervous system indicates that they are not a product of degeneration of a substance or substances contained in nuclei in general. Since all ganglion cells in a given lesion which contain herpetic inclusion bodies in various stages of development, associated with the usual evidences of nuclear degeneration, do not contain them, then the source of their production points rather to a disturbance in metabolic activity of certain injured nuclei. The irregular and bizarre figures which this substance forms, its refractive appearance, its liability, and the fact that it occurs within the nuclei of cells about whose processes a medullary sheath may be formed, suggest that it may be of the nature of the myelins and that the nucleus may be actively concerned in its formation.

The fact that the intranuclear herpetic inclusions occur in their typical form within the same nuclei which contain the above described substance, is another evidence of the specificity of the herpetic bodies; for the presence of the myelin-like substance must represent a peculiar type of degeneration or pathologic activity of these nuclei, not exhibited by other nuclei in which the herpetic inclusions have an identical appearance.

The presence of the above described substance within the nuclei of ganglion cells demonstrates that new substances with characteristic morphology may arise within abnormal cells and are apparently derived from components of the cell, or are built up by abnormal processes within the cell, although structurally they may have nothing to do with an associated virus. This fact makes all the more apparent the importance of exercising caution in the interpretation of the nature of intracellular material in the various virus diseases. If a particular inclusion, however, such as that associated with an

out as sharply stained, orange-red or pink, somewhat refractive masses, round or oval, or as films of irregular contour. Most typically they occur as round or oval bodies, often very numerous, forming a sort of rosette about the herpetic body, or films of this substance may form an imperfect coating over an herpetic body. In early stages of their development the nucleus may appear divided into segments in the center of which one of the bodies lies, the compartments being outlined by threads or granules of chromatin. The round bodies have a characteristic structure. They have a central area which is paler than the periphery and usually contains a node of condensation. When not intensely stained this node is found to be formed by a folding on itself of what appears to be a lining of the body, as if a sphere surrounded by a definite membrane had been ruptured and the lining of the vesicle had coiled upon itself. Looking at it parallel to the axis of the coil, the center appears condensed. These bodies are most prominent in nuclei which present a well developed herpetic inclusion, although they have no structural relation to the herpetic mass and have not been found in association with it except in ganglion cells. Neuroglial and ependymal cells in the same lesion, which contain typical herpetic inclusions, never contain these structures. Nor do all ganglion cells in a given lesion exhibit them. They are to be distinguished by their distinct form and color reactions from the compact, more deeply stained particles of chromatin, and from altered nucleoli, and from deeply stained fuchsinophilic bodies of doubtful origin.

The bodies seem to be composed of a very labile substance and disappear rapidly from the cells following the death of the animal. For a constant demonstration of them the brain of the rabbit in an acute stage of encephalitis must be fixed immediately after death. The following experiment is an example:

76-R. White adult rabbit.

3/1/26 Injected intracerebrally into right hemisphere .25 cc. saline suspension of Ammon's horn from 93-R (fresh herpetic encephalitis).

3/2/26 Temperature 104.3 F.

3/3/26 Temperature 102.5 F.

3/5/26 Temperature 104.9 F.

3/8/26 Temperature 106.0 F.

3/9/26 Temperature 106.0 F.

3/10/26 Found lying on right side, collapsed. Left ear and left eye twitching spasmodically. Temperature 100.0 F.

herpetic infection can occur in cells of various types and preserve its uniformity of structure, the indications are that the virus is immediately concerned in its formations; for the chemical and physical changes which transpire during the disintegration of different kinds of cells no doubt vary widely, as is indicated by the formation of a new substance in the nuclei of ganglion cells and its absence in other types of cells subjected to the same injurious agency.

SUMMARY

1. Intranuclear bodies, distinct from herpetic inclusions, may occur within ganglion cells of the central nervous system in the presence of an acute herpetic encephalitis. This substance may be myelin.

2. Herpetic intranuclear inclusions typical in morphology occur in association with the new substance, and they appear to be specific for the virus whatever other changes the nuclei may undergo.

3. New substances with characteristic form and reactions may appear within injured nuclei in association with a virus, yet have no morphologic relation to the virus itself.

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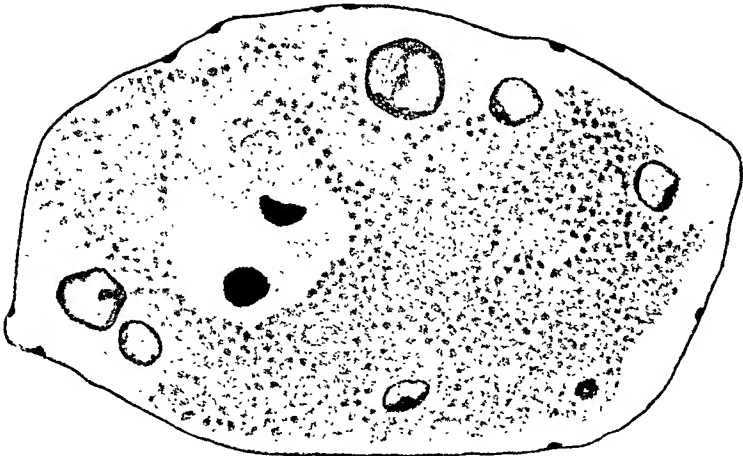
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DESCRIPTION OF PLATES

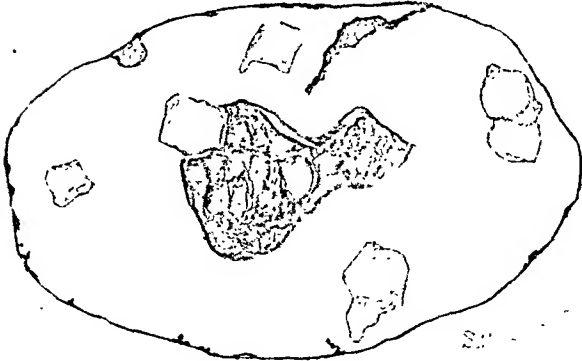
PLATE II5

FIG. 1. Nucleus from a ganglion cell in Ammon's horn. Anilin-fuchsin counterstained with Loeffler's methylene blue. The dark blue masses represent nucleoli, the granular lavender bodies constitute the herpetic inclusion, the spherical red masses are the newly formed material resembling myelin. The chromatin is concentrated in granules upon the nuclear membrane.

FIG. 2. Nucleus of a ganglion cell in Ammon's horn showing a central red-staining herpetic inclusion and several myelin-like inclusions situated in a clear zone between the herpetic body and the nuclear membrane. The section was stained with anilin-acid fuchsin followed by Loeffler's methylene blue.



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tissue-colloids he supposes them to be. From his illustrations one gains the impression that he was studying the delicate reticulum of coagulated protoplasmic material that any histologist would accept as such, but would hesitate to consider as true fibrils. Nevertheless, there is enough interest and weight to Isaacs' theories to demand confirmation or disproof. It will be seen later that his conception of the influence of dehydration on fibril formation is in a measure, borne out.

In studying the fibrous tissue, particularly in healing wounds, the following questions arise: (1) Are these fibrils artefacts? (2) If not, are they preëxisting, or do they represent, colloidal strands that are later coagulated into actual fibrils by such fixatives as Zenker's fluid? (3) Whence do they come, are they produced by fibroblasts or other mesenchymal cells, as the older writers believed, or by precipitation in the intercellular juices or jellies or might they represent prolongations or accretions on the extremities of preëxisting reticulum or connective tissue? (4) What is their relation to fibrin? (5) Is collagen related to reticulin? In the following pages we shall try to answer at least some of these questions.

I. ARE FIBRILS ARTEFACTS?

In order to answer this, we performed some simple experiments. It was noted, in studying healing wounds in rabbits' skin, that the scab contained large quantities of reticulin, and that this mass was connected by a delicate, unbroken reticulum with that of the corium. This network was also intimately interwoven with the epithelium growing over the surface of the wound, the fibrils anchoring this sheet to the underlying granulation tissue.

EXPERIMENT 1. This observation indicated that reticulin might be present on the surface of human granulation tissue, so sterile glass slides were rubbed over the fresh granulations of wounds on a patient's arm, and some of the smears were immediately fixed in Zenker's fluid, while others were permitted to dry somewhat before fixation; the latter showed no morphologic difference. An abundance of fibers and fibrils could be demonstrated, both by Van Gieson's stain and phosphotungstic acid hematoxylin, combined with the Bielschowsky-Maresch silver impregnation. Reticulin was impregnated black, fibrin yellow or violet, and collagen bright vermilion or old-gold. Most of the fibrils took the collagen stain, but some were impregnated with silver (Fig. 1). Under the lens of a microscope, hanging-drops of this exudate were fixed in Zenker's fluid, and nothing was produced which one might call true, new formed fibrils. Preparations were made by simple contact, to avoid mechanical smearing or streaking,

ON THE ORIGIN OF RETICULIN FIBRILS *

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INTRODUCTION

When studying the histopathology of healing wounds, one is always impressed by the fact that the mode of formation of the end-products of this process, the fibrils of the connective tissue, remains a matter of uncertainty. There are still champions of the theory of the intracellular origin of fibrils, and an opposing group which seems to be numerically increasing, that maintains that they arise from the intercellular tissue-matrix. Between these extremes are those who believe that the fibrils are formed outside of cells through the agency of their secretions upon the tissue fluids. In an earlier paper (Foot ¹), the statement was made that collagen fibers were a product of the transformation of fibrin; later (Foot ²), attempts were made to apply this theory to the production of "tubercle reticulum," and it was found that this assumption was erroneous since fibrin and reticulin are produced independently of each other. Baitsell ^{3,4} and Hertzler ⁵ had already published interesting observations on the transformation of fibrin into collagen, as noted in tissue cultures, healing wounds and peritoneal adhesions. Their statements would seem to be quite conclusive, were it not for the fact that they could not be corroborated in the study of the formation of tubercle reticulum. Isaacs ⁶ performed a series of experiments to prove that the fibrils of subcutaneous connective tissue were, in reality, artefacts. He maintained that they could be produced by any physical, chemical or mechanical process that tended to dehydrate the tissue, and based his conclusions upon a series of experiments. His conception of what constitutes fibrils, however, differs materially from that of most observers, and it is probable that, while his conclusions as to the formation of these structures are quite correct, yet the structures that he so names are not true fibrils, but merely the coagulated

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was seen to be composed of a delicate reticulum in which there were numerous webs of fine fibrils (Fig. 3). The crushed muscle showed less shrinkage of its fibers, and the presence of an argyrophil substance (plainly visible in Fig. 3), which had apparently been expressed. The reticulum was coarser, compacted and collapsed (Fig. 4), and lacked the webs seen in the control. One might assume that the argyrophil material had collected in the reticulum and had become impregnated in the same way, thus forming a thicker fiber around the muscle bundle, but one may more easily account for this thickening on the basis of a compacting of the web-like fibrils which were squeezed together in the press. The lymph node showed marked distortion of its reticulum in a plane at right angles to the line of application of force. Thus the experiment of Rössle and Yoshida was completely confirmed.

Our experiments, then, indicate that normal reticulum is pre-existing and not an artefact, that it can be obtained in smears from the surface of a granulating wound, and that it cannot be precipitated in the filtrate of pressed tissues.

III. WHENCE DO CONNECTIVE TISSUE FIBERS COME?

The best way to answer this would be to attempt to grow reticulum and collagen *in vitro*. Since it was always noted that the most abundant reticulum was usually near hemorrhages in granulation tissue and tumors, and as Baitsell had observed fibril formation in living cultures of human blood, an exudate from a rabbit's ear, inflamed with local applications of Croton oil, and rabbit's blood with and without various admixtures of Ringer's solution and fibrogen (tissue fibrinogen) were carefully studied. These cultures were made according to Margaret Lewis's technic⁸ in hanging-drops. They were fixed in formaldehyde vapor, or warm or cold Zenker's fluid. Simple contact preparations were made in order to test out the mechanical effect of streaking. They were allowed to dry before fixation, in order to exclude the possibility of the *reticula* being merely silver deposits in the cracks of dry, caked blood. Much of this preliminary work was disappointing, but we were able to rule out most of these factors as having no bearing upon the production of fibrils and we were encouraged to persevere.

EXPERIMENT 5. Several series of whole-blood cultures were prepared and incubated from one to four days, with controls fixed a few minutes after the drops had clotted. The cultures were fixed either in formaldehyde gas, or warm Zenker's fluid. A drop of 40 per cent formaldehyde solution was run into the concavity of the slide, and the culture was replaced in the incubator until it had become fixed by the vapor; and Zenker's solution, warmed to body temperature,

and fibrils were found as in the smears. The subcutaneous tissue of a rabbit was examined in the living condition in a hanging-drop preparation, and found to contain a dense felting of fibers that showed fraying into fibrils at the free ends; these in the fresh state could be lightly stained with thionin, and the dye produced no new fibrils by coagulation. All of this indicates that fibrils are pre-existing.

EXPERIMENT 2. Further, to check up on Isaacs' experiments, muscle and liver from a freshly killed rabbit were pressed out in a meat press and the juice smeared on slides and fixed in formalin and Zenker's fluid. Ridiculously close imitations of embryonal connective tissue were produced, but only a few fibers that became impregnated with silver. Many doubtful collagen fibrils could be found, but they lacked body and were obviously coagulum. Filtration through filter paper of the juice to which a little normal saline solution was added, resulted in negative smears. Hence the few genuine fibers that were in the unfiltered juice were probably torn out of the tissues and expressed with the fluids rather than precipitated by the fixative. Observation of the action of Zenker's fluid on the unfiltered juice demonstrated an abundant, granular coagulum, but no true fibrils.

II. ARE TISSUE FIBRILS PREEXISTING?

EXPERIMENT 3. Isaacs' experiment, in which layers of tissue were superimposed and fixed in Zenker's fluid, was repeated in the following manner. The twitching pectoral muscle of a rabbit recently killed was folded upon itself, so that its subcutaneous surface should be apposed, and compressed in a meat press in order to bring out as much coagulable juice between the layers as possible. The other pectoral muscle was folded upon itself, but not pressed, and this was then fixed in the same way. According to Isaacs, an unbroken reticulum of fibrils should form between the two layers at intervals, interrupted by clear spaces where the tissue colloids are presumably absent. What we obtained in paraffin sections cut at right angles to the plane of the muscle layers, was quite different, as can be seen in Fig. 2. The two layers of muscle showed fibers extending almost across the gap, sometimes entirely across it, but most of them were truncated so as to reach about half-way, forming a contact line at the middle of the space, which is quite evident in the photomicrograph. Those fibers that extend across the gap do so because they are long enough to reach, but there is nothing to indicate that they are newly formed. The uncrushed muscle showed the same thing, but the gap was wider and no fibers traversed it entirely.

EXPERIMENT 4. In order to answer question 2 more fully, we repeated the experiment of Rösle and Yoshida⁷ by crushing the tissue before fixation, and watching for distortion in the fixed specimens. They used lymph nodes; we used lymph nodes, spleen and skeletal muscle, all quite fresh. We crushed a rabbit spleen with a hemostat in several places, fixed it immediately in Zenker's fluid and made paraffin sections. We crushed living muscle, connected with which was a lymph node, and fixed it in the same way; uncrushed muscle, still twitching and obtained from the same animal, was fixed as a control. Sections of the spleen showed distortion of its reticulum and trabeculae. The uncrushed muscle showed much shrinkage of its fibers away from the sarcolemma which

tirely of laked erythrocytes. After four days, this reticular zone has increased still further in diameter, but the white cells have become very sluggish and have degenerated; in fact, they show marked activity of their mitochondria, and brisk ameboid motion for only five or six hours, after which they become noticeably sluggish. They phagocytize erythrocytes, multiply and bring about laking of the red cells during this period, but they seem to have nothing whatever to do with the formation of the network in which they lie; indeed, they appear to destroy or push aside all the fibrils in their neighborhood, and this gives rise to a ring of denser and more compact fibers around the spaces in which these cells move about. If the plasma or serum spreads out over the surface of the cover-slip, owing to changes in surface tension, the reticulum becomes stretched and torn in such places and long streamers of fibrils fan out into the spreading sheet of fluid, which indicate an apparent elasticity of the reticulum.

OBSERVATIONS ON FIXED CULTURES

After staining the preparations, the following facts are evident: The central mass of erythrocytes remains an opaque mass of blackish disks with yellowish borders, embedded in a matrix of yellowish fibrin. Our interest is focussed on the marginal zone. The reticulum we have described as visible *intra vitam*, now stains in one of two ways; if we are fortunate, it becomes impregnated with silver, and then is gray or black; if not, it takes on a pink stain with the acid fuchsin. The morphology is the same in either event. This variable staining is often noted in paraffin sections and, while it may depend upon some slip in the preparation of the silver-ammonium oxide bath, it probably depends on some other factor: for instance, perfused spleens will often resist impregnation with silver-ammonium oxide or carbonate, but a luxuriant reticulum can be demonstrated in them if silver tannate solutions are employed.

What was described in connection with the living cultures is now confirmed in the stained droplets; the laked erythrocytes prove to be reticulated and, where they are close together, form a finely meshed network that is composed of more or less unaltered red cell reticulum and, where they become distorted or swollen, this reticulum is torn in places and compacted in others. Some of its fibrils still outline red cells, while others are no longer delicate and faint,

was run into the concavity with a dropper, and the culture was carefully immersed in the fluid. As soon as it became firm and yellow, the culture was floated face down in Zenker's fluid in a Petri dish. If the serum was dropped directly on the surface of cold Zenker's fluid, it would spread out over the cover-slip and coagulate into strands that stained and resembled collagen; when the cultures were lowered carefully into warm Zenker's fluid, no change was noted.

These drops of rabbit's blood were placed on cover-slips ringed with vaseline, so that they usually floated face down on the fixative, which prevented the formation of precipitates; if they would not float, a little vaseline was added at the corners. The fixed cultures were stained as follows: If Zenker's fluid was the fixative, they were treated with weak alcoholic iodine to remove the mercury, 5 per cent sodium thiosulphate to remove the iodine, and 0.25 per cent potassium permanganate and 5 per cent oxalic acid to remove the chromates. Five minutes submersion in the first, 1 in the second, 5 in the third and 10 in the fourth was sufficient. When formalin was used no such treatment was necessary. The cultures were next impregnated with 2 per cent silver nitrate for 48 hours and then treated with silver ammonium oxide, 5 per cent formalin, 1 per cent gold chloride and 5 per cent sodium thiosulphate according to the Bielschowsky method (Foot⁹). Van Gieson's stain was used as a counterstain, either with or without Harris' hematoxylin. The resulting preparations showed a central mass of black erythrocytes and yellow fibrin, with a peripheral zone of pinkish fibrillae and grayish or blackish laked corpuscles and fibrils. All cultures were observed immediately after they were prepared and at frequent intervals thereafter, on a warm stage *in vivo*. They were killed by fixation immediately after they had clotted, and at 24, 48 and 96 hours thereafter, and stained as above noted.

OBSERVATIONS ON LIVING CULTURES

When freshly made, the cultures consist of a central drop of blood too dense to examine with the high power, and a peripheral zone where the plasma has spread out somewhat over the cover-slip. This marginal zone is our field of observation. At first one notes a progressive laking of the erythrocytes in this zone, apparently caused by their contact with the glass and the alterations in the plasma due to the action of the air contained in the concavity of the slide. Fenn¹⁰ has discussed this phenomenon at length. A few polymorphonuclear and mononuclear leucocytes soon appear at the edge of the culture and, after a few hours, large numbers of them are crawling about. At this time one sees that the laked erythrocytes are linking up to form a finely meshed network which borders the blood drop at its periphery and forms a delicate fringe. After twenty-four hours this fringe is well developed and very evident, and portions show refractile, more or less straight fibrils. Fibrin, which is abundant in the center of the droplet, apparently plays no part in the production of this reticulum which seems to be composed en-

similar to those we obtained in our cultures. Where the erythrocytes were crushed and rolled, the reticulum was compacted into jet-black filaments like those in the culture reticulum. It was quite evident that this was not dependent upon the presence of fibrin, for we obtained similar pictures in the defibrinated blood smears. Smears of washed corpuscles showed a reticulum in the cells that had become flattened out on the slide.

DISCUSSION

With all these data, let us consider what we have accomplished toward answering the questions postulated at the beginning of this paper.

1. *Are fibrils artefacts?* We can answer this in the negative, so far as reticulin fibrils are concerned, but with collagen fibrils the matter is somewhat different, for we have seen that coagulation of the blood serum and tissue juices in Zenker's fluid may give rise to structures closely resembling collagen fibrils.

2. *Are fibrils preëxisting?* From our experiments on crushing fresh tissues before fixation and from the results of the examination of fixed smears from granulation tissue, we can state that reticulin is preformed. Again we are in doubt as to the collagen.

3. *Whence do fibrils come?* We have shown that the reticulin fibrils in our cultures result from the disintegration of erythrocytes, and a rearrangement of the reticulin they contain, always basing our conclusions on the supposition that the silver impregnation is specific for reticulin. It seems that this is a viscid material which, when freed from its associated hemoglobin, becomes rearranged into longer, straighter fibrils by stretching and pulling. This force is exerted during life, however, and has nothing to do with the manipulation of dead, or fixed tissue. At first the reticulum is readily identifiable with that of the erythrocytes, later it is only by tracing its development from this finer network that we can be sure whence it originated. This we have done *in vivo*, and checked our results by staining after fixation.

It is strange that the reticulum of the erythrocyte is so unfamiliar to the average pathologist. Meves¹² described it more than twenty years ago in connection with amphibian blood, and Ruzicka¹³ investigated it in the mammalia. Cupp's article gives a beautiful and convincing description of the reticulum of amphibian and mammalian erythrocytes, including those of man; it was published over eleven years ago.

but stouter, straighter and sometimes somewhat beaded. They closely resemble true reticulum. Figs. 5 and 6 show respectively the more compact, finely-meshed reticulum and the spaces that surround the macrophages. As one approaches the periphery of this network, the meshes grow progressively coarser (Fig. 7) and, where the drops have spread out as already described, the filaments no longer bear any resemblance to the red cell reticulum, but course out over the cover-slip in long streamers. Here and there the reticulum of the erythrocytes loses most of its morphology and appears to be a tangled mass. The coarser reticulum is depicted in Fig. 8.

As all this very definitely pointed to the erythrocytes as the origin of the reticulum in these cultures, another experiment was performed in an attempt to study the red cell reticulum.

EXPERIMENT 6. About 2.5 cc. of the heart's blood of a rabbit were drawn into each of two test-tubes, to one of which was added 0.25 cc. of fibrogen and to the other a like amount of Ringer's solution. The clots were incubated for 24 hours, and were then carefully removed from the tubes, sliced, and fixed in Zenker's solution; the serum was smeared out on slides and likewise fixed in this fluid. The clots were mounted in paraffin, and then both sections and smears were impregnated with silver and counterstained with Van Gieson's stain. Some of the former were also stained with hematoxylin and eosin, Van Gieson's stain, and phosphotungstic acid hematoxylin.

Smears: These show erythrocytes more or less completely laked and impregnated with silver so that their reticulum is readily demonstrable. It is quite similar to that described by Cupp¹¹ in his paper on the structure of the erythrocyte. He employed a different technic, but the pictures that we both obtained are identical (Fig. 9).

Sections: The erythrocytes in our sections are not laked, and little or no structure can be made out at their centers, but in the silver impregnations, the periphery of each cell shows a black ring with nodular thickenings. These correspond to the nodes and short rods seen in the uncut erythrocytes of the smears. By searching, one can identify the domed surfaces of cells that lie more or less intact in the section, and focussing up and down on these, a reticulum can be discerned lying upon the surface of the cell (Cupp's "cell capsule").

EXPERIMENT 7. In order to rule out fibrin as a factor in the formation of reticulum in our cultures, we added fibrogen to one tube in the preceding experiment without producing any change in the red cell reticulum. To exclude it still more definitely, we took blood from a rabbit, smeared some of it on slides for a control and then defibrinated the rest. We smeared some of this defibrinated blood on slides and then washed the remainder in Ringer's solution, making smears of the washed corpuscles. We then impregnated the smears with silver and counterstained with Van Gieson's stain. The result in these smears was not so satisfactory as was that in those obtained from the serum of the clots in the preceding experiment; the hemoglobin obscured the reticulum, but the latter was still demonstrable, and the smear of whole blood presented figures strikingly

above, but it does not explain why the separated fibers are argyrophil, while the coarser, compact bundles are fuchsinophil.

While the reticulum produced in cultures of blood, *in vitro*, has a very similar appearance to that of the lymphoid tissue and, more particularly, of granulation tissue and tumors of the connective tissue group, it would be false presumption to claim that observations made upon these blood cultures could explain the production of normal reticulum. It would be difficult to apply a theory involving the wholesale destruction of erythrocytes to the histogenesis of reticulum in general. A novel aspect of fibril formation has, however, been presented by these experiments and it is not unlikely that it has a very close relation to the processes that take place in granulation tissue and tumors; for these usually present a good deal of hemorrhage and diapedesis of erythrocytes, and there is undoubtedly more reticulum in the neighborhood of extravasations of blood than elsewhere.

In closing, it would be well to point out that cells other than erythrocytes appear to contain rather similar intracellular networks which might conceivably be deposited in the tissues by a lysis of the unformed residuum of the cell during processes of a retrograde nature.

The pronoun "we," employed extensively throughout this paper, refers to my co-worker Mr. Marvin C. Mènard, to whom I am much indebted for assistance in performing the various experiments detailed above.

CONCLUSIONS

1. Reticulin fibrils are not artefacts.
2. They are preformed, and probably of a viscid nature; it is probable also that they become firm and possess some tensile strength as they grow older.
3. Fibrils closely resembling reticulum are produced in hanging-drop tissue cultures of blood by the disintegration of erythrocytes and a rearrangement of their intracellular reticulum.
4. They bear no discoverable relationship to fibrin.
5. Collagen is, apparently, a chemical substance in the tissue juices that impregnates fibrils or fibers composed of protoplasm, rather than the sole constituent of the white, fibrous tissue.
6. These conclusions apply to the reticulum of granulation tissue,

As this reticulum is demonstrable with silver impregnations of fixed, laked erythrocytes, and as we have followed its elaboration from a delicate network into a coarser, more fibrillary reticulum in blood cultures, the conclusion that the latter is the direct product of the former in this experiment is inevitable. Whether or not this is true in the body is a matter for speculation. Fibrin stains yellow with Van Gieson's stain, and would be readily recognizable were it present in the marginal reticulum; however, it is absent from the very beginning, appearing to retract into the central clot as it is formed.

4. *What is the relation of connective tissue fibrils to fibrin?* We have answered this in the preceding paragraph. There appears to be no demonstrable relationship between the two in our cultures.

5. *What is the relation of collagen to reticulin?* We thought that reticulin preceded collagen in the formation of collagen fibers, the latter being transformed from reticulin into collagen. Our experiments have shown this to be only in a measure true; from what has been said, it will be seen that collagen is possibly a colloidal, or semi-fluid substance that can be coagulated into granules or into the semblance of fibrils by the action of fixatives, notably Zenker's fluid. It seems likely, from our observations, that this substance impregnates various fibrils or tissue strands, rather than forming fibrils of its own accord. Thus fibrils could become impregnated with collagen and lose their affinity for silver, staining with acid fuchsin. In the same way, fibrin could form a matrix which might become impregnated with collagen, or become "collagenated"; and even the protoplasmic processes of cells, or the reticulum of erythrocytes might conceivably undergo a like transformation. This would explain the appearance of fibrils that stain partly with acid fuchsin and become impregnated with silver, and appear blackened elsewhere; one frequently encounters such particolored fibers in granulation tissue and in tumors. Hertzler noted that the fibrin in peritoneal adhesions rapidly lost its affinity for picric acid and became fuchsinophil in Van Gieson preparations. Our hypothesis would explain this observation very well.

Mallory¹⁴ has recently said that separated fibrils of connective tissue are argyrophil, while compact fibers are fuchsinophil, the two being essentially the same material, considered chemically. This statement does not conflict at all with what has been said

PLATE 118

- FIG. 5. Oil immersion photomicrograph of the reticulum at the margin of a culture. The meshes are fine and the relation of the culture-reticulum to that of the erythrocytes is fairly evident.
- FIG. 6. Oil immersion photomicrograph of the transition zone between unlaked erythrocytes and culture reticulum.
- FIG. 7. Oil immersion photomicrograph of the transition zone between fine-meshed and coarse-meshed reticulum at the margin of a culture. Note the comparative scarcity of white cells in these pictures.

PLATE 119

- FIG. 8. Oil immersion picture of the long "streamer" fibrils at the extreme periphery of a culture. Figs. 5 to 8 were taken in almost unbroken progression from the edge of the mass of erythrocytes to the outer margin of the surrounding "reticulum."
- FIG. 9. Erythrocytes in a smear of serum from a blood clot that was incubated twenty-four hours. Rabbit blood. Note the intracellular reticulum in the erythrocyte center of the field.

All photomicrographs made with the assistance of Mr. J. B. Homan, of the Department of Medical Art, of the Medical College. The stain used is the Bielschowsky-Maresch-Van Gieson combination described in this paper.

as well as to that produced *in vitro* in blood-drop cultures; they may also apply to *tumor reticulum* and *tubercle reticulum*, but no claim is made that a definite solution of the problem of the histogenesis of fibrous tissue in general has been presented by these experiments.

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DESCRIPTION OF PLATES

PLATE 116

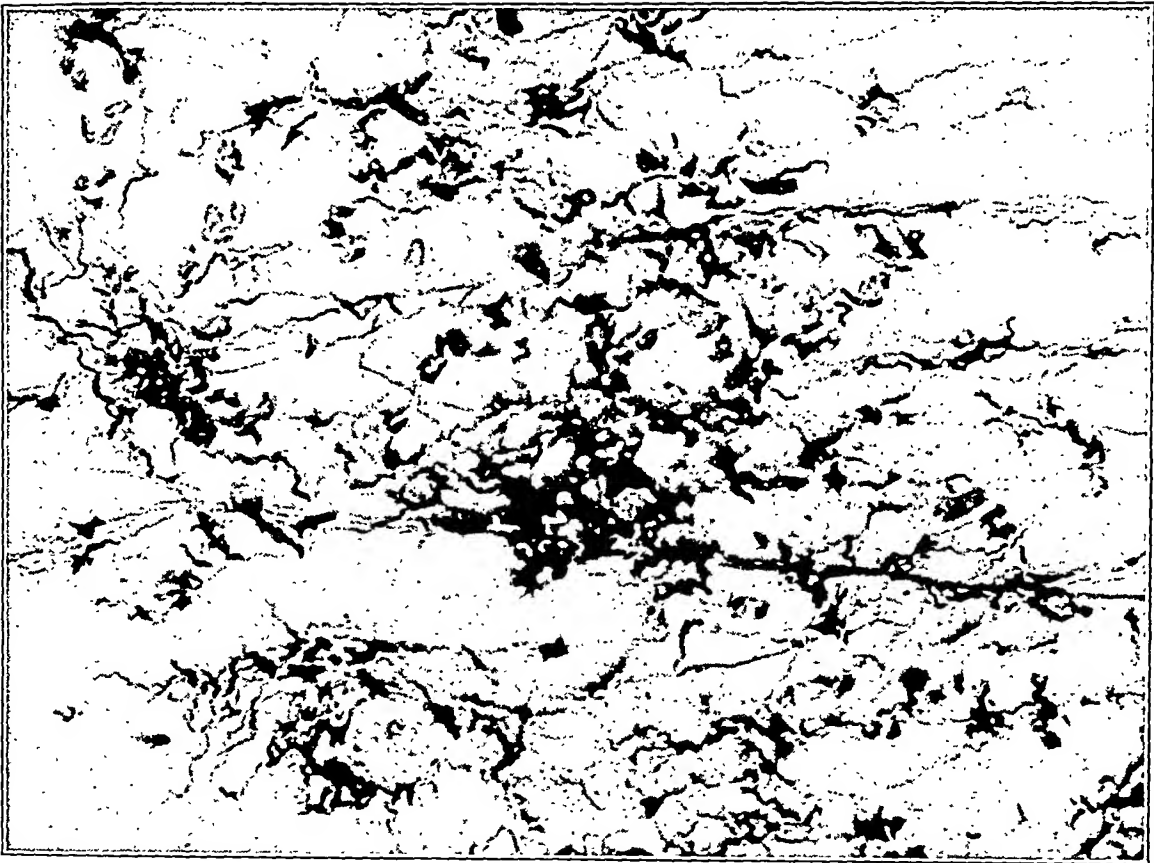
FIG. 1. A smear from fresh granulations on a wound, high power photomicrograph. The coarse, diffuse fibrils are collagenous, the delicate strands mostly reticular.

FIG. 2. Low power photomicrograph of two apposed layers of rabbit muscle after crushing together in a meat press. Note that in most instances the fibrils in the gap between the masses of muscle run only to the middle. There is no newly formed coagulum of fibrils.

PLATE 117

FIG. 3. High power photomicrograph of uncrushed, normal rabbit muscle. Note the webs in the interfascicular reticulated membrane and the argyrophil substance in the muscle bundles.

FIG. 4. Same power, muscle crushed in meat press. The interfascicular webs have disappeared and the argyrophil substance in the muscle has been apparently expressed. The reticulum is coarser, thicker and somewhat distorted in one plane.



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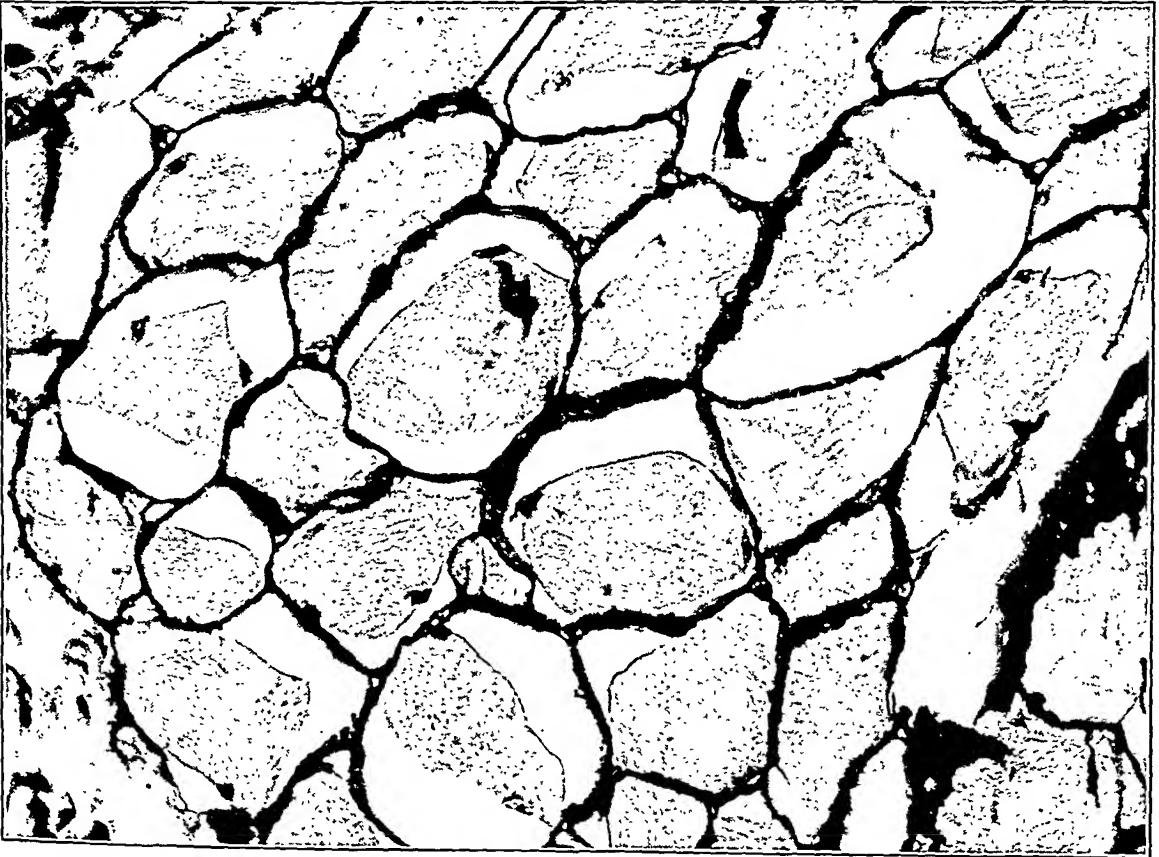
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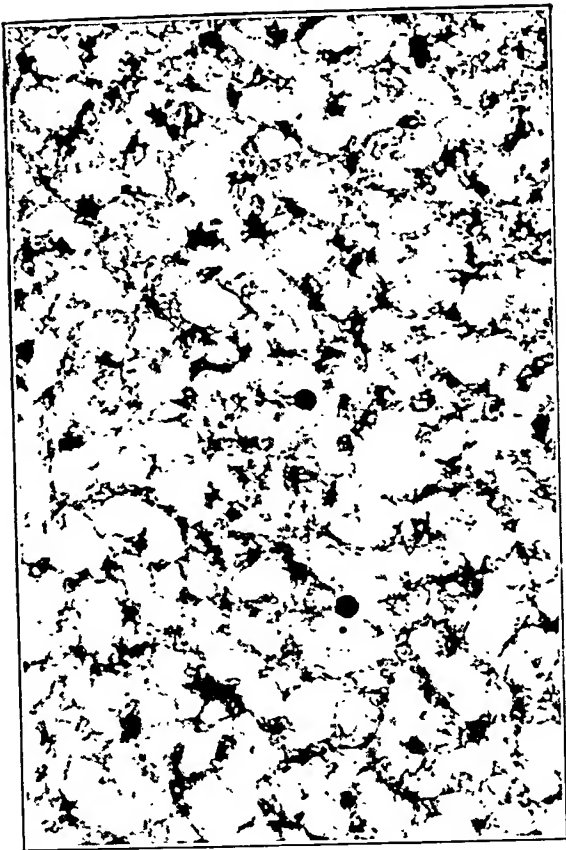
On the Origin of Reticulin Fibrils



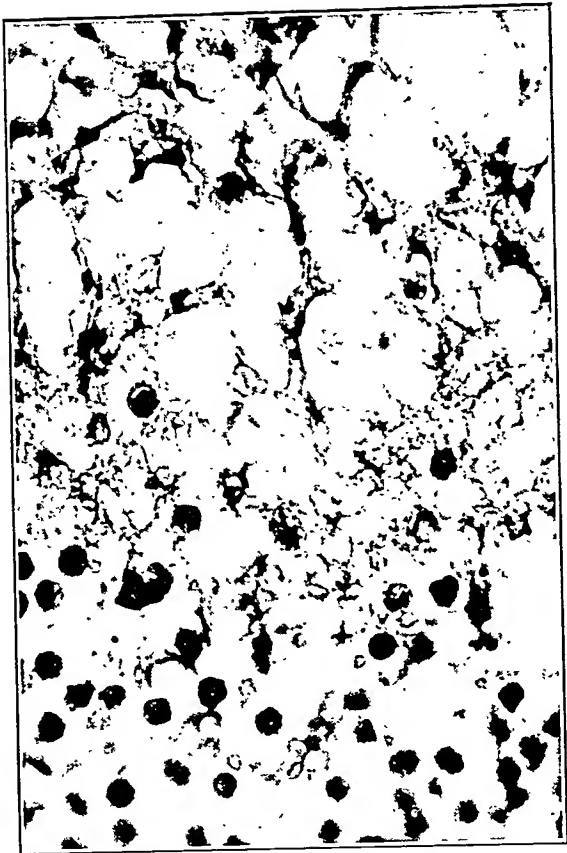
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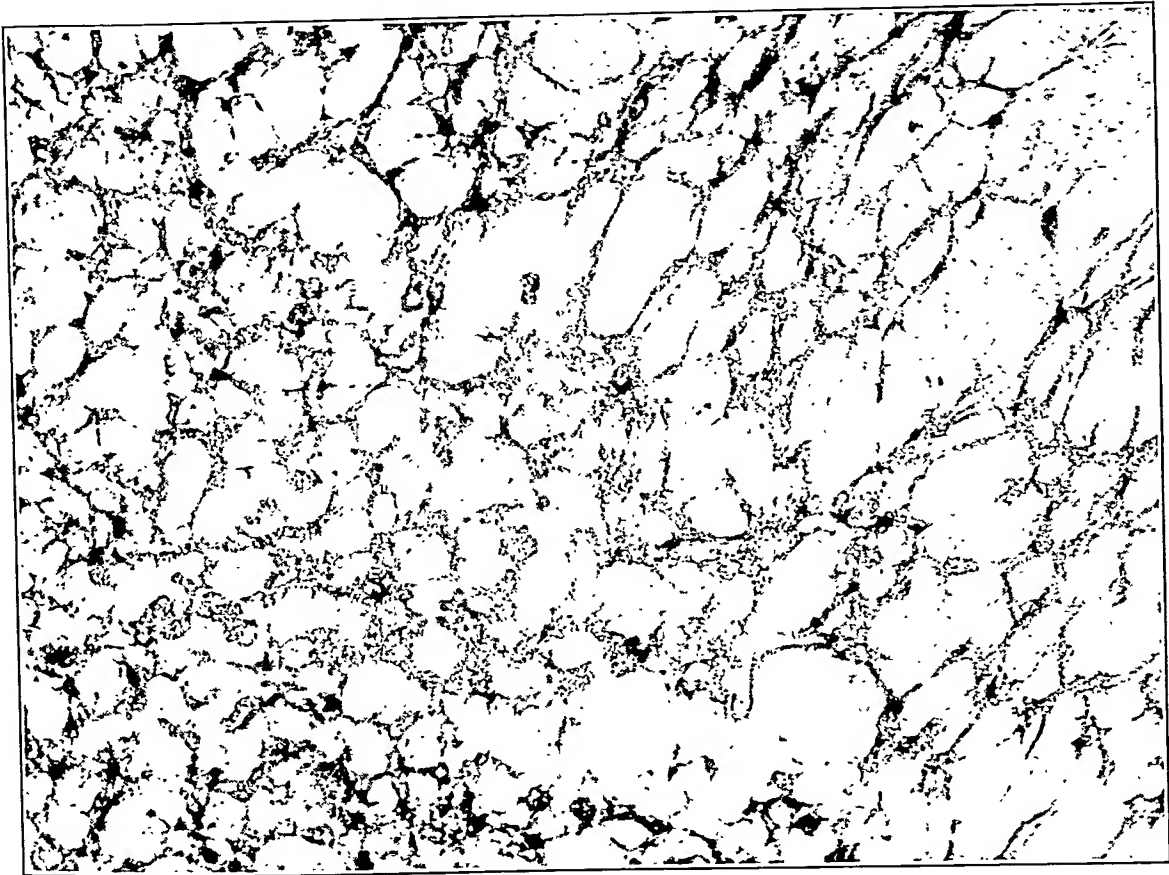
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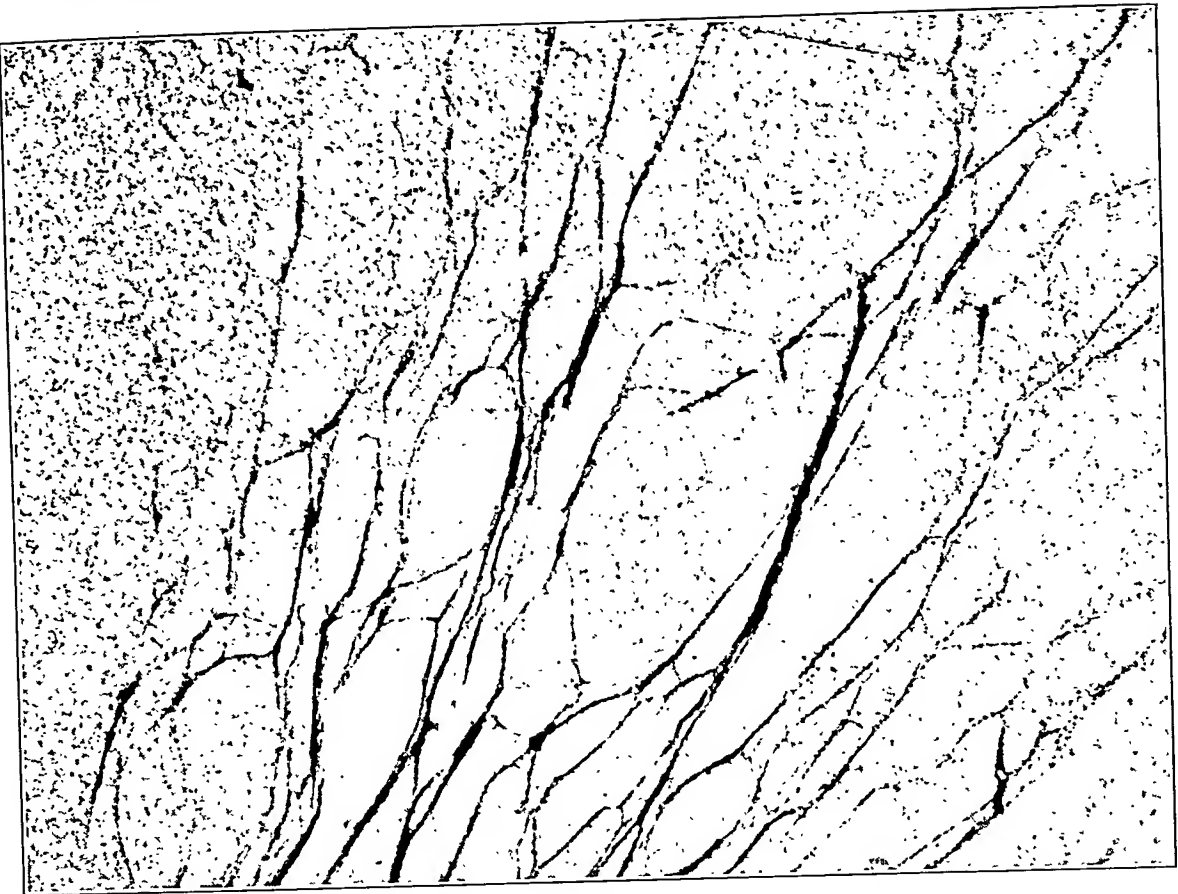
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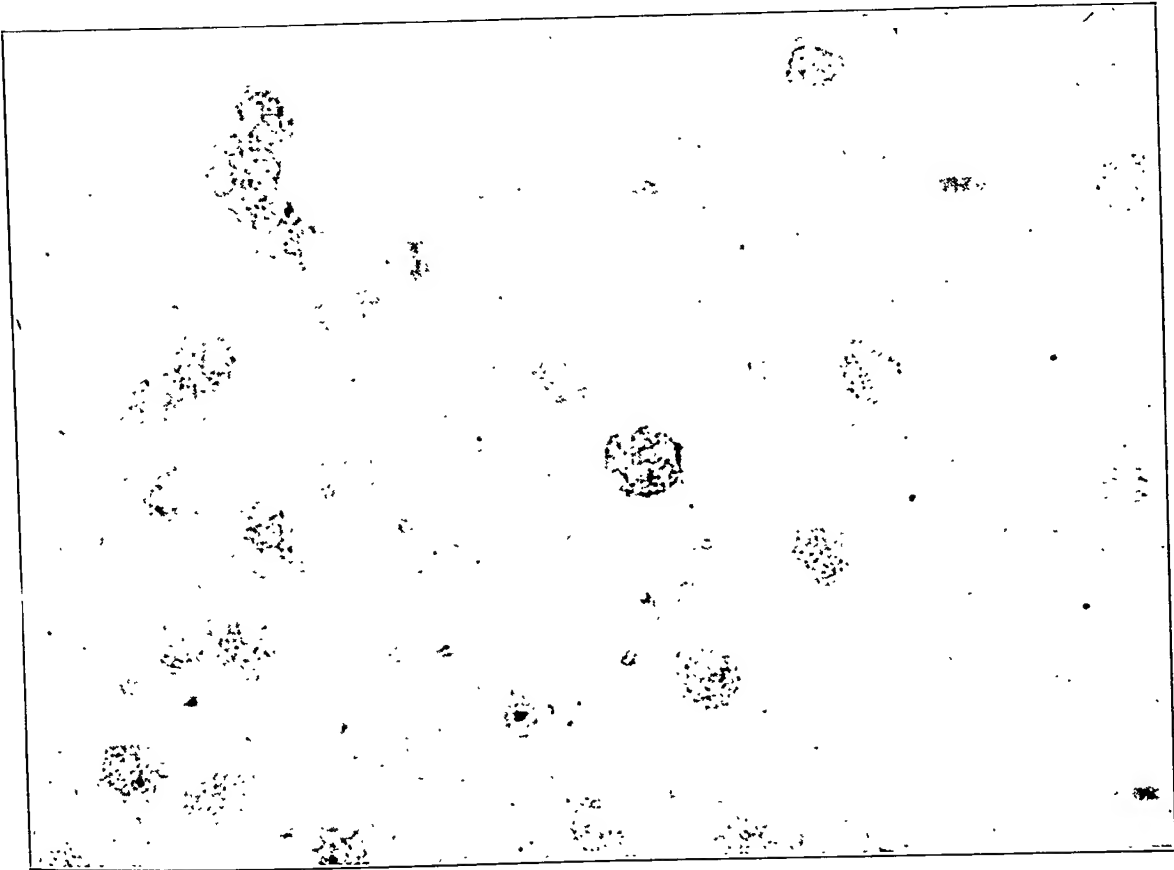
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STUDIES ON ENDOTHELIAL REACTIONS

CORRECTION

In Vol. III, No. 3, page 282, first line of second paragraph, for "There is an identity of the individuality differentials," read "An identity of the individuality differentials, that is, a complete loss of the individuality, has not yet been reached within the inbred families."

pendently and using somewhat different methods, came to the conclusion that the pulmonary dust cell was derived from the capillary endothelium of the lung. We believed that the capillary endothelium became swollen, its cells proliferated and then migrated into the alveoli, there to become free phagocytes and to multiply still further by mitotic division. We suggested that the phagocytes already present in the alveoli under normal conditions (not called out by a special crisis, such as the injection of dyes or bacteria) had

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the same origin, presumably migrating from vascular endothelium from time to time in order to form a sort of scavenging reserve force. We both described cells, which Lang ('25) has subsequently named "septum cells," that are normally present in niches or recesses in the alveolar wall and considered that they had the same origin.

Our theories have been critically studied. They have been accepted either entirely or partially by some and rejected by others. Many investigators agree as to the mesenchymal origin of these cells, although questioning their being derived from the capillary endothelium. A number have put forth well substantiated claims that they arise in the circulating blood as monocytes, migrating to the alveoli as occasion arises. M. R. Lewis ('25) and her pupil Eliot ('25) have employed vital staining to show that the phagocytes are at least of mesenchymal origin, whereas Wislocki ('24) agrees, but remains in doubt as to their precise derivation.

Thus we might be drifting back toward the monocytic origin of these cells, as formerly postulated by Metschnikoff ('07), were our regression not impeded by numerous papers, chiefly by German investigators, who claim that they are merely somewhat altered, desquamated alveolar epithelium. Metschnikoff said: "For long, the large 'dust cells' of the respiratory channels were looked upon as being epithelial cells which were capable of taking up carbon particles, microorganisms and other foreign bodies. In reality these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi." The most recent article to support the epithelial side of the argument is by Gross ('27) from Aschoff's laboratory. It will be well to quote at some length from his paper, for in this way one may present the subject in an unbiased fashion, as the statement emanates from the opposing camp and will, for that reason, stress their views rather than ours.

Résumé by Gross: "The ancient dispute concerning the origin of the epithelioid cells has not come to rest since the time of Baumgarten ('01) (fixed tissue cells) and Metschnikoff ('88) (leukocytes). Herxheimer ('03) has injected into the debate, as a third possibility, the participation of the alveolar epithelium. The Japanese school (Kiyono, '14) traces them to histiocytes. Töppich ('25), whose experiments were performed to evaluate Herxheimer's work, champions the endothelial derivation. He sees, as early as one hour after infection, a migration of swollen capillary endothelial cells through

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STUDIES ON ENDOTHELIAL REACTIONS

X. ON THE ORIGIN OF THE PULMONARY "DUST CELL" *

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INTRODUCTION

It would be very inadvisable to attempt to present the results of the experimental work to be reported in this paper without first reviewing the subject as a whole. So much controversy has centered about the mononuclear phagocytes in general and the pulmonary dust cell in particular that the reader would probably be bewildered by a discussion concerning the latter, were he not permitted to familiarize himself with the views of the various disputants. It will, therefore, be the purpose of this article to afford him this opportunity by presenting a full review of the present status of the doctrine of the dust cell; after which, he may proceed to an enlightened consideration of the value of the data that constitute the original portion of the paper.

Seven years ago, Permar ('20) and Foot ('20), experimenting independently and using somewhat different methods, came to the conclusion that the pulmonary dust cell was derived from the capillary endothelium of the lung. We believed that the capillary endothelium became swollen, its cells proliferated and then migrated into the alveoli, there to become free phagocytes and to multiply still further by mitotic division. We suggested that the phagocytes already present in the alveoli under normal conditions (not called out by a special crisis, such as the injection of dyes or bacteria) had

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In his summary there is one misstatement that should be corrected; Lang does not deny the existence of alveolar epithelium. He says: "The epithelium is of no importance either, as the alveolar wall, according to my previous investigations, is not provided with any special 'respiratory' epithelial layer, *except the non-nucleated plates.*" The italics are mine. Kageyama's work is not entirely relevant to the discussion, as tuberculosis in the lungs of rats and mice presents an altogether different picture from that which is observed in the case of other mammals.

Here, then, is a statement from the "epithelial camp," whose adherents antedated Herxheimer's "injection" of the epithelial origin of the dust cell into the debate by a number of years, as is indicated by Herxheimer himself. This older literature will be found listed in his paper, in Permar's and in mine ('20).

The Experiments of Westhues and Westhues: Gross merely touches upon the work of the Westhuses ('22, '25), who also adhere firmly to the epithelial origin of the dust cell and have done careful and excellent experiments in an endeavor to prove their case. It has been considered in a former paper (Foot, '25), but it should be mentioned here. In view of the fact that Wislocki ('24) has stated that "Chinesische Tusche" is a colloidal and not a mechanical suspension of carbon as I had thought, it would be well to withdraw my criticism of the work of H. Westhues, wherein I stressed the point that particulate carbon would not mark capillary endothelium.

Westhues noted that there was a decided predilection on the part of the alveolar phagocytes for carbon, carmine being taken up sparingly and slowly. From this he concludes: "As phagocytosis in the lungs takes place more rapidly and energetically than histiocytes could phagocytose, it follows that the engorged (vollgefressenen) cells in the alveoli are not emigrated histiocytes, but could only be alveolar epithelial cells." But, has it been proved that the alveolar epithelium is so energetically phagocytic? Have not the Kupffer cells and splenic phagocytes marked phagocytic properties? More recently H. and M. Westhues ('25) performed clever and painstaking experiments to prove this point. They believed that Permar's inferences, drawn from lung sections that had necessarily been cut in many planes, were "very daring." They attempted to narcotise the phagocytes, but without success. Next they replied to my earlier criticism by using colloidal Elektrokollargol for intratracheal

the reticulum of elastic fibrils into the alveolar lumen and their transformation into large mononuclears and epithelioid transitional cells. 'There can be no question of alveolar epithelium, so far as the great majority of the cells is concerned, for disregarding transition-pictures, exactly the same cell types may be recognized as lying definitely within the capillaries.' He explains the circumstance that he found no alveolar epithelium at all by the fact that these cells, 'probably under the direct action' of the large doses of bacilli, had been totally destroyed. Kageyama ('25) found bovine and avian tubercle bacilli, injected in massive doses into the peritoneal cavity, appearing in the pulmonary blood as soon as two hours thereafter. The excretion of these bacilli into the alveoli followed very promptly. After eighteen hours, changes were first noted in the lung tissue, consisting of a proliferation of the alveolar epithelial cells which phagocytosed the bacilli and desquamated into the alveolar lumen as in desquamative catarrh. He never found bacilli in the endothelial cells.

"Tissue cultures have also been drawn upon to solve the problem. Timofejewsky and Benevolenskaja ('25) discovered that tubercle bacilli inhibit the growth of rabbit lung tissue cultures. Despite the fact that they established that the particularly active elements in the cultures were the epithelial cells, they do not venture to exclude monocytes, endothelial cells and fibroblasts from the category of bacillary phagocytes, but believe that the epithelioid cells of an organism develop rather from the connective tissue than from the alveolar epithelium. Lang ('25), who denies the existence of alveolar epithelium, finds exudative and productive processes in similar experiments. Pagel ('25) has attempted to answer the question as to the origin of the exudate cells in caseous pneumonia on morphologic grounds. 'In the guinea pig, unmistakable transition pictures as well as structural identity, permit us to trace the exudate cells to the alveolar lining.' Proof in the case of the human subject has fallen flat. ('Ist gescheitert.')."

Further on Gross states: "In the case of the rabbit, I must hold fast to the theory of a primary epithelial reaction." His reason for this is based on the flimsiest of morphologic evidence obtained from the examination of experimental animals a month to six weeks after the injection of tubercle bacilli or dust into the trachea. His argument might be epitomized in the statement: "The dust cells look like epithelium, therefore they are epithelium."

was, in reality, mitosis in monocytes adhering to the capillary walls on the inside.

It is apparent that one can neither prove nor disprove much in this connection unless the process be observed *in vivo*, or many methods be applied simultaneously. M. R. Lewis ('25) has observed carbon-laden monocytes leaving the pulmonary capillaries to enter the alveoli of the lungs of living frogs; she mentions, however, that this animal does not normally possess dust cells. Herzog ('24) has also noted carbon-laden sessile cells in the capillaries of a frog's tongue detaching themselves and floating off into the circulation, or migrating through the vessel walls and wandering to points quite distant therefrom. Of course, these "sessile cells" may have been clinging monocytes and only apparently connected with the capillary endothelium.

That even observations *in vivo* are subject to misinterpretation is shown by the fact that Stilwell ('26) has repeated Herzog's experiments in Maximow's laboratory and has been unable to confirm them. She finds that the vascular endothelium does, indeed, store ink for a time, indulging in what she calls "passive phagocytosis," but this ink is gradually transferred to the perivascular tissue while under direct observation. Furthermore, the ink-laden cells that leave the vessels in the frog's tongue are, according to her views, monocytes; she did not observe any rounding-up of the vascular endothelium to produce monocytes or polyblasts. This is of interest not only because it shows Herzog to be in error, but because it entirely refutes my former claims as to the specificity of ink for vascular endothelium and the argument that the cells seen to migrate from the vessels were therefore detached vascular endothelium. In the face of such observations, made *in vivo* and therefore quite different from deductions derived from the study of fixed tissue, one can only retract one's claims and admit that they are unsound.

Thus we come to a point where the matter seems to require some method other than the mere observation of sections of fixed tissue, be they ever so carefully made, for its solution. Wislocki ('24) has considered the origin of the dust cell to be impossible of detection by this means. He says: "We find it impossible from sectioned material or by the methods employed for its identification, to determine its origin." Employing supravital staining with neutral red and Janus green and dyeing the scrapings of fresh lung from

and Elektroferrol for intravenous injection. No phagocytosis was observed on the part of the capillary endothelium. This would appear to be an important point, were it not for the fact that the endothelium was presumably healthy and would therefore scarcely phagocytose foreign material. They then perfused rabbit lung with normal saline solution to wash the circulation clear, injected 1:150 India ink into the trachea and suspended the lung in warm saline solution in an incubator for half an hour. The ink was taken up avidly by the dust cells. Reversing the process, they flooded the capillaries with ink and the alveoli with normal saline solution, observing no phagocytosis of the ink by the endothelium or the dust cells. In the first instance no ink was shown to have been phagocytosed by the respiratory epithelium *in situ*, either in the description or illustrations; in the second, although it is stated that there was no phagocytosis of the ink by the endothelium, the colored illustration depicts black granules all through the walls of the alveoli and embedded in the violet tinted cytoplasm of their cells. It is difficult to reconcile this unfortunate discrepancy with the text of the article.

Theories of Mesenchymal Origin of the Dust Cells: So much for the epithelial origin of the dust cell, let us now consider the other side of the argument. When Permar and I published our experimental results we were content to avail ourselves of the evidence at hand; perhaps it was insufficient. My line of deduction was as follows: Cells were found in the alveolar spaces in tuberculous inflammation containing carbon that had been administered intravenously. It appeared as fine particles and coarser clumps in what was interpreted as capillary endothelium which was swollen and which showed mitotic figures in what were supposed to be its lining cells. Therefore the intra-alveolar phagocytes were emigrated cells from the capillary walls. Experimentation with meningeal tuberculosis (Foot, '22) subsequently proved that there was little change in the local capillary endothelium in that case, yet tubercles formed near the vessels and consisted of carbon-marked cells. Similar cells were found within the lumina of the pial vessels, often in mitosis and mingled with the other blood cells. Obviously something was wrong with the earlier theory, which should have applied here as well, or this was a notable exception to the rule. It is possible that what Permar and I interpreted as vascular endothelium in mitosis,

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acteristics of the monocyte and histiocyte when treated supravivally with neutral red and Janus green or stained with other dyes. The beautiful color plates correspond very closely with Wislocki's description of the cells observed in lung scrapings. As Masugi's work was undertaken "at the suggestion and under the direction of" Professor Aschoff, it would seem that Wislocki had satisfactorily complied with the criteria for identifying monocytes and histiocytes as laid down by the latter. This will be of great importance later on in this article.

Fried ('27) has just published a report in which he apparently denies the presence of the alveolar epithelium altogether, but a careful perusal of his paper brings out the fact that he, too, concedes the existence of non-nucleated epithelial plates. According to him, what appears to be respiratory epithelium is, in reality, masses of histiocytes that are attached to the alveolar wall near the angles and which, when stimulated by the presence of foreign material, become detached to give rise to the typical dust cell, or "Herzfehlerzell." His most striking results were obtained in rabbits by injecting large doses of pyrrhol blue in 1 per cent concentration in Ringer's solution intratracheally, either in one massive (acute) dose, or in five smaller (chronic) doses of 5 cc. each. Because the cells attached to the alveolar wall gave a typical picture of vitally stained histiocytes, he believes that they are of mesenchymal and not of epithelial origin. His conclusions are necessarily based upon morphology alone. The epithelium of the bronchi, although drenched in the dye, remained unstained; the vascular endothelium of the pulmonary capillaries was also unstained; he could demonstrate no respiratory epithelium and therefore he disbelieves in its existence. He rules out the monocytes as parent cells because of the total lack of histiocytic proliferation in the other organs. Well founded though these conclusions may be, they leave loop-holes for the critic in so far as they fail to explain the histogenesis of the groups of mural histiocytes, and overlook the possibility of independent propagation of the monocytes while circulating in the blood stream. His failure to demonstrate respiratory epithelium was due to his technic, for it may be easily demonstrated with silver impregnations.

Having surveyed the literature in a fairly comprehensive fashion, one must admit that the subject is still unsettled, as both Gardner ('26) and Sacks ('26) remark in recent reviews on this topic. If there

rabbits and a cat, after a preliminary intravenous injection of Higgins' ink, he found that the various pulmonary elements could be identified and classified. The leucocytes, erythrocytes and monocytes were easily recognized; the dust cells were found to be roughly divisible into three classes: Type (1) large cells with oval nuclei and a variable number of large neutral red vacuoles, Type (2) slightly smaller cells with many carbon granules and a smaller number of red vacuoles, Type (3) large cells which, in addition to a few specks of carbon and the neutral red vacuoles, contained large, greenish, refractile granules arranged about the periphery of the cells. The carbon charge was always in inverse ratio to the number of neutral red vacuoles, the inference being that the cells preferred carbon to neutral red; just as in Westhues' ('22) experiment it was found that they had a greater affinity for carbon than for carmine. The epithelium was often found in sheets or cords, it was partly of the non-ciliated cuboidal and partly of the ciliated cylindrical type. Its cytoplasm was filled with minute red granules. None of the epithelial cells contained carbon. Transitions between "clasmatocytes" (dust cells) of Types 1 and 2 were observed.

Wislocki concludes: "The carbon deposited in the capillaries of the lung is gradually eliminated probably by way of the circulation, the respiratory tract and the lymph channels. After the initial phase of deposition of carbon particles in the lungs, phagocytic cells play a prominent part in its storage and elimination. By studying the fresh, living cells of the lungs in a warm-box, it has been shown that the carbon particles are phagocytosed principally by clasmatocytes. The origin of these cells in the lungs is discussed." As to this point, he says: "Three possibilities suggest themselves. The first is that it (the dust cell) arises by mitosis from the endothelium of the pulmonary capillaries. The second is that it arises in the liver and spleen and is carried by the circulation to lodge in the lungs. The third possibility is that it arises in the stroma of the lungs from clasmatocytes normally present there." In explanation, it may be said that "monocytes" are Aschoff's "blood-histiocytes," the "large mononuclear leucocytes" of clinical parlance; while "clasmatocytes" are the "tissue histiocytes" of Aschoff, "endothelial leucocytes" of Mallory, "polyblasts" of Maximow, etc.

An article by Masugi ('27) appears in the same number of "Ziegler's Beiträge" as does Gross' paper; it describes the tinctorial char-

a rabbit and 25 cc. of its heart's blood were withdrawn after a wait of fifteen minutes into a syringe containing 2 cc. of 10 per cent aqueous sodium citrate. After mixing this well, 8 cc. were injected intravenously into each of three rabbits. These next received intratracheal injections of from 3 to 4 cc. of pasteurized milk containing 0.5 per cent of saturated alcoholic Sudan III. The milk was administered slowly through an aspirating needle introduced directly through the skin into the trachea, an influx of air bubbles into the syringe indicating that the needle had penetrated to the lumen. The rabbits stood the injection well; there was transient dyspnea with some regurgitation of milk, but after a minute or two they were on their feet again and quite alert. By the next day they seemed perfectly well.

One rabbit was killed 24 hours, the next 48 hours and the third 90 hours after the injection, by introducing air into the circulation. Necropsy revealed lungs of an almost normal external appearance in the case of the first two rabbits, but section showed some consolidation at the bases, and slices from these sank in water. Nevertheless, the consolidated lung lacked the congestion and granular appearance of the usual pneumonia. The lungs of the third rabbit were in no way grossly remarkable.

As each rabbit was killed, the chest was opened, the lungs and heart removed *in toto* and bits of lung were either squeezed out or scraped off on slides coated with neutral red and Janus green or Nile blue sulphate. Coverslips were sealed over the drops with paraffin-vaseline, a little Ringer's fluid being added if the material was too scanty to cover the whole coverslip, and the preparations were incubated for a few minutes at body temperature before being examined microscopically on a warm stage. The slides were prepared by adding 40 drops of 1 per cent neutral red in absolute alcohol and 30 drops of Janus green in like solution, to 10 cc. of absolute alcohol, flooding clean slides and allowing them to dry. The Nile blue sulphate was used undiluted in 1 per cent solution in absolute alcohol. Before the blood from the donor rabbit was injected into the others, it was examined to determine that there was no free carmine in its plasma.

After these films were completed, the lungs were injected through the trachea with 10 per cent neutral formalin until moderately distended and dropped entire into that fixative. Part of the material

be a way in which it may be settled, it would appear to lie along the lines of: (a) Supravital staining, (b) intravital or supravital injection of dyes, (c) by the discovery of a specific method for staining the dust cell and its parent cell in fixed tissues. Therefore the work detailed in the remainder of this paper has been undertaken along these three lines. For a more general consideration of the ramifications of the reticulo-endothelial system the reader is referred to reviews by Aschoff ('24), Foot ('25), Gardner ('26), Sabin ('22) and Sacks ('26).

REPORT OF ORIGINAL EXPERIMENTAL WORK

In order to attack this problem from several angles at once, the following experiments were performed. Rabbits were given intravenous and intratracheal injections of various dyes and of milk, the cells that responded in the alveoli were examined *in vivo* in supravital films, the lungs were sectioned after freezing or after embedding in paraffin, and examined from the morphologic standpoint. A specific method for identifying monocytes, histiocytes and dust cells on the one hand and distinguishing them from epithelium or from pleural mesothelium on the other, was fortunately found. Material from scraped human lungs was examined supravitally as soon as permissible postmortem and the sputum from a patient with aortic insufficiency was investigated in supravital films. Lung tissue from similar cases was sectioned in paraffin and studied with routine stains and the specific silver tannate technic. The findings with the latter were checked up with material from fresh human spleen and a case of tuberculous meningitis. Fresh human spleen was also examined in supravital films and the findings confirmed those obtained with the silver technic.

EXPERIMENTS ON RABBITS

Technic (Experiment No. 1). The first experiment combined the technics of Eliot ('26) and Wislocki ('24). Two-tenths gm. of carmine rubrum optimum (Coleman & Bell) were ground in a mortar until fine and stirred with 6 cc. of distilled water, added little by little, until the mixture was smooth. It was centrifugated for ten minutes to throw down the coarser particles, boiled and cooled to body temperature. Four cc. were then injected into the ear vein of

these stains. Nile blue sulphate would also act as a fat stain and indicate whether the neutral fat underwent chemical changes after becoming incorporated in the cells.

RESULTS IN SUPRAVITALLY STAINED FILMS

These confirmed Wislocki's findings in every particular, his three types of "clasmatoocytes" were recognized and their striking similarity to Masugi's illustrations was at once noted. The results with Nile blue sulphate were as satisfactory as those with neutral red; this dye gives more rapid impregnation of the granules and the pictures are sharper. Reviewing the types of cells noted in both stains, but omitting a description of the neutral red pictures, — as Wislocki has already covered this, — we find the following:

Dust Cells: These were large, rounded or ovoid cells with numerous dark blue to greenish granules which varied not only in color, but also in size and shape. There were fine, sharply stained granules and large, variably stained vacuoles which sometimes contained a speck or two of dust. Some of these cells showed uniform dark blue granules with a pale, yellowish, unstained nuclear space; others showed the variable characteristics noted for dust cells in general, while a third type — the largest of the three, contained large, polyhedral, refractile pieces of yellowish or brownish material as well, sometimes almost as large as erythrocytes. Frequently there were large vacuoles in the third type that contained either erythrocytes or drops of neutral fat, which stained rose with the Nile blue sulphate. The mitochondria seen in the neutral red-Janus green technic were not prominent in this case. Sometimes one occasionally observed carmine-colored granules, but as they were also present in films from controls that had received no carmine they probably represent metachromatic staining. Attraction-spheres were not prominent. The carmine injected in the first experiment could not be found in the blood cells of the animals that received the transfused blood. This is not readily explained, for the only differences in the technic, as compared with Eliot's, were the use of sodium citrate instead of heparin and a slightly longer delay in withdrawing the blood. The second experiment demonstrated that this delay could have made little difference in the results.

Monocytes: Small monocytes were frequently encountered in the stained films; a little larger than polymorphonuclears, they were

was sectioned on a freezing microtome and part embedded in paraffin and subsequently sectioned at 7 microns.

(Experiment No. 2.) This practically duplicated the first, but five cc. of the carmine suspension were injected intravenously into the donor rabbit and its blood was withdrawn five, instead of fifteen minutes later. Only two rabbits received this blood intravenously and only 1 cc. of milk (this time diluted one-half with Ringer's fluid) was injected into their tracheae. The supravital examinations were omitted in this case and the lungs were removed *in toto* 24 and 48 hours after they had been injected with the milk. In neither case was there anything abnormal to be noted, the organs being of normal color, consistence and general appearance.

Donor Rabbits: The donor for the first experiment died about a week after giving its blood. No gross evidence for its death was manifest at necropsy. Its lungs were removed and injected with neutral formalin, as in the other cases; its spleen was also fixed for microscopic examination.

The donor for the second experiment was killed by air-embolus 96 hours after the bleeding and its lungs (which appeared in no way abnormal, although in this case the donor rabbit had also received a milk injection) were fixed as above. Bits of spleen and liver were also secured for examination as to the distribution of the carmine.

PURPOSE OF THE EXPERIMENTS

By introducing "marked monocytes" from another animal, it was hoped that the blood origin of the intra-alveolar phagocytes might be proved or disproved; if these contained carmine it is obvious that they would most probably represent marked cells from the donor animal — unless polymorphonuclear leucocytes should have emigrated and been engulfed by the dust cells, in which case uncertainty would result. The milk was used as a mild irritant, to excite the emigration of macrophages into the alveolar sacs; incidentally, the neutral fat would serve as a dye if it retained the Sudan III, if not, use could still be made of it by staining with more of that dye or using Nile blue sulphate. Ballou and Ballou ('27) have used this method for tracing the fate of lipiodol in the lung. The purpose of the supravital stains was to determine the behavior of the dust cells, the epithelium, vascular endothelium and mesothelium toward

in small fenestra in the membrane; these were probably "septum cells" seen from above.

Mesothelium: Sheets of pleural tissue were quite unstained with Nile blue sulphate, although very fine, closely arranged and refractile granules which stained faintly with neutral red could be observed in their cytoplasm.

Free Fat: This was present in the form of larger or smaller drops of light orange or rose, according to the stain. Apparently most of the Sudan III in the milk had disappeared.

FROZEN SECTIONS; SUDAN III STAIN

(*First Experiment*)

These showed that the dyed milk injection had caused a mild lobular pneumonia in which the exudate was composed of polymorphonuclear leucocytes and macrophages in about equal numbers; the greatest reaction was seen in the lung of the two-day rabbit. After four days the lungs had practically returned to normal, but there was still a large number of dust cells in the alveoli. As we are interested chiefly in the fat in these sections, let us confine our attention to that substance. It lay free in the air-sacs at first and was then taken up by the phagocytes, the epithelium being merely dusted with fine particles. The large cells protruding from the alveolar septa (considered to be epithelium by Aschoff, Gross and Westhues, and histiocytes by Kiyono, Fried and Gardner), contained much fat. In the frozen sections there was little, if any clue as to the origin of these cells. The monocytes in the capillaries, the polymorphonuclears to a lesser degree and the vascular endothelium all showed intracellular fat and there appeared to be some of it free in the blood plasma, although this may have been scattered out in the process of sectioning. Most of it was contained in dust cells and monocytes. Occasionally globules were found between the bronchial epithelial cells, but their cytoplasm was practically free from Sudanophil material, although diffusely "rusted" by the stain. There was a moderately heavy deposition of fat in the peribronchial lymph nodes, some within phagocytes and some apparently free.

The areas of bronchopneumonia in the first two rabbits showed such distortion of the normal pulmonary architecture that very little could be judged as to what had taken place; apparently the walls

usually filled with blue granules, uniform in size and brilliantly stained, with the untinted nucleus crowded to the periphery. A large proportion of these showed a cluster of granules in the concavity of the reniform nucleus, with a more thinly distributed line of granules extending like the horns of a crescent to the nuclear poles; a comparatively unstained zone separated them from the cell periphery (Figs. 1 and 2). The appearance of these cells, when stained supravitaly with neutral red and after fixation with Sudan III and silver tannate, is shown in Figs. 4, 5 and 6. Figure 3 shows a monocyte with a phagocytosed fat droplet, transitional between Figs. 1 and 2 and Fig. 7, which represents a "Type 3" dust cell. Figure 8 is a similar transitional type impregnated with silver tannate and containing a fat vacuole.

Polymorphonuclears: These stained a diffuse light blue and showed yellowish granules, with a few that were blue.

Lymphocytes: They took on an even, diffuse light blue color though unstained as to nucleus and granules.

Vascular Endothelium: Bits of capillaries were included in the scrapings (Fig. 10), with a few erythrocytes in their lumina. Nothing indicated that they stained specifically, nor were the fibroblasts at all granular.

Epithelial Cells: Three types were recognized:

(1) Large, flat or slightly curled plates with no nucleus or merely a shadow of one (Fig. 9).

(2) Groups of interlocking, flanged cells, some of them showing denser nuclear shadows and resembling the descriptions of Bremer ('04), Ogawa ('20) and Stewart ('23).

(3) Non-ciliated cuboidal, or ciliated cylindrical cells from the bronchial mucosa.

None of these cells stained deeply, the larger cells showed a diffuse light blue, but no granules (very fine granules were seen with neutral red), while the small bronchial cells either stained not at all, or only faintly with an occasional minute granule of Nile blue sulphate. Some of the groups showed a drop or two of rose-colored neutral fat in, or on, their cytoplasm. Branching, Y-shaped streaks were observed in the large epithelial plates, corresponding to Stewart's pictures of epithelial mitochondria. Sometimes, where there were extensive sheets of epithelium which had separated from the alveoli, one might observe vitally-stained, rounded cells lying

and in the fat-treated lungs they tended to take on a somewhat reddish tinge.

Here, then, is a means of impregnating dust cells specifically. As there was a possibility that this depended upon the presence of ingested fat, lungs from six other rabbits were sectioned and impregnated in the same way, invariably showing the same thing although presumably fat-free. The dust cells, monocytes and polymorphonuclears were the only cells that became specifically impregnated. By combining supravital intratracheal staining with neutral red, Niagara blue 3b or Nile blue sulphate injected at the time of death from air-embolism and incubated *in situ* in the dead rabbit for fifteen minutes, as recommended by Gardner ('27), slight variations in the color reactions of the impregnation were effected, but the granular impregnation remained the same. As the fixation was ordinary neutral formalin and the routine paraffin technic was used, instead of Gardner's more elaborate procedure which requires an adjustment of the pH of the fixative necessitating special color indicators or a potentiometer, the results with neutral red were inferior to his. The cells showed the typical granular stain seen in supravital films, however, but the bright red of these had paled to a dull brick-red.

In order further to check up on the specificity of the silver tannate method, smears of rabbits' blood were made, fixed in neutral 10 per cent formalin and impregnated. The monocytes and polymorphonuclears showed the specific dark brown cytoplasm and sepia granules seen in the sections, although the monocytes tended to be paler in the smears. The lymphocytes were pale and showed no granular stain, their cytoplasm was almost colorless and their nuclei stood out distinctly, as with iron hematoxylin. The erythrocytes were either slightly and diffusely brownish, or where they had dried and laked somewhat, showed blackish reticulation. This reticulation was observed in some of the sections also.

Observations in Silver Tannate Sections: In the sections from the first milk-injection experiment, monocytes were found crowding the capillaries, undergoing karyokinesis while within their lumina, and dividing within the alveoli after emerging from the vessels. A monocyte is shown in a large vein in Fig. 11. Careful scrutiny of all the slides failed to demonstrate any coarse granules in the epithelium, mesothelium or vascular endothelium. The large cells that

of the alveoli had become thickened by engorgement of the capillaries and proliferation of the stroma, and exudate had filled the air-sacs here and there. There were fields of large, pale cells liberally dotted with fat, closely resembling those in Fried's photomicrographs, but there was little evidence to show whence they came. These might have been histiocytes, proliferated adventitial cells, thickened capillary endothelium or migrated monocytes — that they were epithelium, however, was most improbable as they lay outside of the air sacs, rather than within them.

The only assistance obtained from the use of Sudan III, then, is the fact that it is taken up by the phagocytes in a manner that stains them in the same way that either neutral red or Nile blue sulphate does. The granules, vacuoles and foreign material are found to correspond accurately in all three methods, therefore there must be a striking similarity in the chemistry of these stains within the cytoplasm.

PARAFFIN SECTIONS

(First Experiment)

These differed very little from the frozen sections. As the fat had been extracted by the chloroform, the pictures were somewhat less complicated. No carmine could be definitely identified, although many leucocytes and dust cells contained minute granules that appeared to give off a reddish luster under the oil-immersion lens. The experiment, then, completely failed to corroborate Eliot's observations.

Silver Tannate Impregnations of Paraffin Sections: (First experiment.) As cell granules were well demonstrated by silver tannate impregnations used in experimenting with the Rio de Horta technic (Foot, '27), it was supposed that this method might aid in solving the dust cell problem. As a result, a very satisfactory means of identifying monocytes and polyblasts was discovered. The sections impregnated with silver tannate showed that the dust cells became reddish brown, with brownish black to sepia granules that corresponded in every way with those observed in the supravital films and the Sudan III sections. Furthermore, these granules were usually grouped in rosettes or balls as in typical monocytes. No other cells in these sections except the polymorphonuclears showed similar characteristics; these had similar, but rather finer granules,

mixture of equal parts of pasteurized milk and Ringer's solution, injected in much smaller quantities, caused only a local reaction in the bronchi. There was no pneumonia. No carmine-marked cells were found in any of the sections, it seemed as though none had been transferred from the donor rabbit, despite the fact that it had a larger intravenous injection of carmine suspension than did the first donor.

FINDINGS IN DONOR RABBITS

The donor rabbit of the first experiment showed relatively little carmine in the lungs and a good deal in the spleen. In the former it was contained in monocytes and polymorphonuclears within the capillaries and very occasionally within a dust cell in which it appeared as a group of carmine particles that occupied about as much room as would an erythrocyte. In the spleen the carmine was in large macrophages which, owing to the fact that the rabbit was found some time after its actual death, also contained the oxydase granules that one finds in somewhat "spoiled" formalin-fixed tissue. These were brownish black and corresponded in their arrangement with the vital granules seen in supravital, Sudan III and silver preparations. These were also present in the monocytes and polymorphonuclears of the capillary sinuses and larger vessels, where more or less carmine was also encountered.

Two points are brought out in this case: The spleen had apparently acquired most of the injected carmine. Certainly it contained more than the lungs, and the fact that dust cells were found in the air-sacs, laden with carmine that had been introduced intravenously, strongly indicated that this particulate material had been carried out of the vessel within the cytoplasm of emigrating monocytes, rather than transferred from the circulation to intra-alveolar dust cells. If the latter hypothesis were true, the carmine would have to traverse the vascular endothelium, stroma and alveolar epithelium. Furthermore, no carmine was found free in the alveolar spaces.

The donor rabbit in the second experiment, killed by air-embolism four days after bleeding, showed some carmine in macrophages in the spleen and a trace in the Kupffer cells of the liver. None was found in the lung. Although this animal had some milk injected into the trachea, the lungs were practically normal.

Silver tannate impregnations of the liver and spleen showed the

are attached to the alveolar septa appeared in the rôle of greatly swollen, clinging monocytes; smaller monocytes might be seen congregated in the capillaries and apparently emerging therefrom (Fig. 12). There was a general dotting of the various pulmonary elements by a light silver precipitate, but this was totally different from the sharply defined and definitely grouped granules in the monocytes or dust cells (Fig. 13). In properly impregnated sections the epithelium of the alveoli could be observed as it lined the extremity of an air-sac and one could discern the epithelial cells and non-nucleated plates, and the capillaries and reticulum beneath them (Fig. 14). The latter was an immense help in keeping the topography of the lung clear in the infiltrated areas. The epithelium could be better brought out in the sections where neutral red had been injected at the time of death. It was seen to correspond accurately with the descriptions of Ogawa and Stewart, except that my preparations showed fenestrations in the epithelial membrane, often occupied by intercalated dust cells. Of course, the empty fenestra might have been fixation artifacts, but the presence of dust cells in some of them, both in these sections and the unfixed scrapings observed *in vivo*, makes this quite unlikely.

Where the alveolar walls had become much thickened in the areas of lobular pneumonia, one noticed two things: The capillaries were distended with monocytes and polymorphonuclears and their endothelium had become vague and merged with the stroma, so that wide fields of pale cells might be observed, with here and there a monocyte lying in the tissue spaces. The cells of these fields resembled vascular endothelium, adventitial or connective tissue cells, — pale, swollen, with ovoid and vesicular nuclei and vacuolated, reticulated cytoplasm. They showed no definite granules other than an occasional blackish grain that might have been precipitate (Fig. 15). On more than one occasion dust cells were observed to be enveloped in veil-like epithelial plates, as though these had been lifted off by them and carried into the alveolar space, where they remained folded about the cell that had detached them.

FINDINGS IN SECOND RABBIT EXPERIMENT

Attention was focussed upon paraffin sections stained with hematoxylin alone or impregnated with silver tannate. The observations tallied in the main with those of the preceding experiment, but the

in Fig. 16. Here again, one found the epithelial plates wrapped about the dust cells. Comparing the hematoxylin and eosin controls with the silver impregnations, one noted that the former showed dust cells heavily laden with hemosiderin and carbon ("Herzfehlerzellen"), but totally lacking the smaller, uniform black granules that were so prominent in the silver impregnations (Fig. 17). Another case of chronic passive pulmonary congestion with infarcts was examined and the granular impregnation of the dust cells was far less striking; this was in material that had been fixed some time postmortem and proves that one should be sure of the freshness of one's material before drawing conclusions adverse to this technic. It has been found to be quite worthless in brain tissue that has remained unfixed for a day or more. Even shorter periods are unfavorable to the specificity of the stain in warm weather.

Spleen and Brain: Paraffin sections from a human spleen and from the meninges in a case of tuberculous meningitis show that the monocytes, polymorphonuclears and polyblasts in these become impregnated exactly as in the case of lung tissue. The splenic endothelium of the capillary sinuses differs absolutely in its staining properties from these cells. The reticulo-endothelium is so vacuolated, so intimately associated with fibrils, and its granules so indefinite, that it also differs from them to a certain extent.

SILVER TANNATE TECHNIC *

Cut thin paraffin sections from formalin or Zenker-fixed material and remove the paraffin in the usual way. If Zenker's fluid has been used, remove the mercury by treating the sections for five minutes in mahogany-brown, alcoholic iodine solution. Bleach in 5 per cent aqueous sodium thiosulphate. Wash. Remove the chromium salts by five minutes treatment in 0.25 per cent potassium permanganate and ten minutes in 5 per cent oxalic acid, washing between solutions. Wash and re-wash in distilled water. Mordant for fifteen minutes in a solution of 0.15 per cent pure tannic acid, 3 per cent ammonium bromide and 10 per cent neutral formalin; this should be done in an incubator, first heating the mordant to 55° C. Treat for thirty

* It is advisable to summarize this procedure here, although it is described in full elsewhere (Foot, '27).

specific granular stain in the Kupffer cells of the former and in monocytes circulating in the sinusoids; the spleen was sprinkled with monocytes, also showing the specific granules. These findings further strengthen the supposition that this method marks monocytes specifically, and they point out the fact that the Kupffer cell is, indeed, different from ordinary endothelium. It takes a granular impregnation quite similar to that seen in the monocyte, which apparently indicates a close relationship between the two. Are Kupffer cells, for instance, merely monocytes anchored to the sinusoidal endothelium by one or more pseudopods?

EXAMINATION OF HUMAN MATERIAL

Supravital Films of Lung Scrapings: Scrapings made from lungs in cases of chronic passive congestion were treated exactly like those from the rabbit lungs; the cases chosen for examination were necropsied as soon after death as permissible. In lungs removed as long as eight and fourteen hours postmortem, the results resembled in every detail those obtained in the case of rabbits. The cells were still viable in both instances and were observed on a warm-stage, the staining of the nuclei being considered a criterion of cell death. The same types of epithelium, stroma, blood cells and dust cells were observed; the latter were plainly marked with the carbon that abounds in Cincinnati atmosphere.

Supravital Films of Sputum: Fresh sputum from a case of cardiac decompensation was obtained from the wards on two occasions and examined by the supravital method; again the results were quite similar, although the copious and tenacious mucus interfered rather noticeably with the efficacy of the neutral red. Nile blue sulphate, however, still gave good results, and the check-up on the observations of necropsy material was perfectly satisfactory.

Paraffin Sections from Human Lung: Specimens of lungs showing chronic passive congestion were sectioned and stained with hematoxylin and eosin as a control, and the silver tannate impregnation was used for critical observation. They showed, even more strikingly than the rabbit lung, the specific character of this impregnation for dust cells. These were very dark, filled with small, uniform black granules and they stood out in bold relief in comparison with the desquamated epithelial cells whose cytoplasm showed no granules and was rather vacuolated and violet-gray. This is well shown

fail to demonstrate any similarity between epithelium, whether alveolar or bronchial, and the dust cell, but actually separate them into two distinctly morphologically unrelated groups.

2. Dust cells often appear in the lymphatics and lymph nodes of the lung laden with carbon, tubercle bacilli, dyes, fat or other material that has come in through the trachea or circulation. There they remain. Permar ('23), Haythorn ('13), Sewell ('18) and Foot ('20) have all reported this phenomenon. Is it more likely that these are cells of mesenchymal origin, or strangely metamorphosed epithelium? Sewell manifested a great deal of difficulty in reconciling the latter view with his findings and his explanation was not, even then, at all convincing. He was forced to regard these as renegade epithelial cells that had become transformed into leucocytes.

3. Macrophages or polyblasts are found in large numbers in a variety of processes in epithelial organs, but apparently it is only in the case of the lung that their presence is ascribed to epithelial proliferation and desquamation.

4. It has been proved that the epithelioid cells of hepatic tuberculosis are formed directly from the Kupffer cells (Evans, Bowman and Winternitz, '14; Goldman, '09, '12; Oppenheimer, '08 and others. To-day no one would consider the bile duct or hepatic epithelium as a source of epithelioid cells in tuberculosis of that organ. As the Kupffer cell appears to be somewhat different from the ordinary vascular endothelium, its participation in the formation of tubercles may be misleading to those who maintain that the vascular endothelium produces the macrophages, as I once believed.

5. Tubercles identical with these arise in the spleen and lymphoid tissue where phagocytes in every way similar to dust cells abound and where there is not epithelium to produce them.

6. Typical multinucleated syncytia, or giant cells, are formed from the dust cells in the alveoli; this is not characteristic of epithelium, which forms multinucleated cells usually under neoplastic conditions, but these differ materially from the typical foreign body giant cell.

7. Stewart ('23) describes the mitochondria of alveolar epithelium, when stained by the Altmann method, as rod-like, often branching or Y-shaped. Such structures may be seen in the epithelial cells in supravital films of scraped lung. I have pictured them in Figure 9. Stewart also shows a mature epithelial cell "about

seconds with three drops of strong ammonia to 100 cc. of distilled water, while the sections are still warm. Impregnate for five minutes in silver-ammonium oxid prepared as follows: To 10 cc. of 1 per cent silver nitrate add one drop of 40 per cent sodium or potassium hydroxid, dissolve the precipitate in five drops of strong ammonia (which should leave a few grains still out of solution), dilute up to 200 cc. with distilled water. Use two baths if the first becomes turbid after two or three minutes. Wash in distilled water and "reduce" for two minutes in 20 per cent neutral formalin. Wash at the tap. "Tone" for two minutes in a 1:500 solution of Merck's "brown, acid" gold chlorid, in which 0.5 per cent bichloride of mercury has been dissolved with the aid of heat. Wash at the tap and fix in 5 per cent sodium thiosulphate (Hypo) for two minutes. Wash and dehydrate in the usual manner with alcohol of increasing percentages, xylol, and mount in Canada balsam.

If the sections be too dark, they may be lightened by immersion in strong potassium cyanid solution (aqueous), but it is better to run through a new set of sections and increase the strength of the ammonia wash, used after the mordant, to ten drops instead of three to 100 cc. of distilled water. Tissues vary a good deal and one must do a certain amount of experimentation on each batch of slides in order to produce the best results. The depth of impregnation may be largely controlled by the strength of the ammonia wash — a weak wash producing dark sections, a strong one lighter impregnation.

DISCUSSION

We come, finally, to a consideration of this subject as a whole in the light of the evidence of others which is, perhaps, somewhat intensified by the additional data supplied by the experiments just described. The easiest way to undertake this task will be to set down *seriatim* the various hypothetical sources of the dust cell and to discuss each in turn.

Epithelial Origin

As the arguments in favor of this have been set forth at the beginning of the paper, let us consider those opposed to it. There are several valid reasons for rejecting the epithelial theory.

1. Supravital staining with neutral red and Nile blue sulphate and the apparently specific silver tannate impregnation not only

Personal observation indicates that the finding of typical rosettes, which should mark the monocyte as such, is more or less a matter of chance; they may or may not be present. In fixed tissue they seem to occur rather more regularly than in fresh films. Be this as it may, it seems that the weight of opinion is tipping the scale in the favor of the monocyte as the parent of the dust cell.

A very telling point in favor of the monocytic origin is the great rapidity with which these cells appear in the alveoli. The Germans have used this as proof of their epithelial origin, as desquamation could readily account for such a phenomenon, but it does not account for the radical difference in morphology between epithelium and dust cells when properly stained. Migration of monocytes from the capillaries is not open to this criticism, for not only do they exhibit the same general morphology, but they will (if kept alive *in vitro*) actually become indistinguishable from dust cells. This has been noted by Carrell and Ebeling ('22), by the Lewises, by Gardner and in my latest experiments. Mitosis may be observed in monocytes within pulmonary capillaries and in dust cells, which would add to their rapidity of production and, although not entirely accounting for it, would indicate that two closely related types were multiplying in response to the same stimulus.

The origin of the monocyte is for the embryologist to establish; once in existence, however, it seems that this cell is capable of independent self-perpetuation, without drawing on any particular organ or tissue reservoir to repair the inroads of an inflammatory process on the supply at hand. Removal of the spleen does not lessen the supply of monocytes in any way (Foot, '23) and the hypothesis that they are the progeny of the extremely specialized endothelium of the venous sinuses of that organ is contradicted by the striking difference in the morphology of the two types and, even more so, by the fact that splenic sinus cells do not stain at all in supravital films. This I have just determined by experiment on fresh human spleen.

For years Maximow has been championing the lymphocytic origin of some of the "polyblasts" in the organism. His "polyblast" is essentially the same as the macrophage, clasmatocyte or endothelial leucocyte. His theories have been sharply criticised and I ('25) have been one of the critics. He has recently published experiments ('27) that effectually disarm this criticism, as he reports

to desquamate" into the alveolus; the striking change in its granules is at once apparent; instead of rod-like mitochondria, we see spheroidal granules of varying size. The cell corresponds accurately with a swollen monocyte or dust cell; was he not picturing one of these in this instance? No Y-shaped or rod-like mitochondria are seen in the dust cells, which differentiates them sharply from epithelium.

8. Finally, the arguments of the supporters of the epithelial origin of the dust cells are based exclusively on morphologic similarity, chiefly in hematoxylin and eosin preparations or similar sections. This similarity, under these circumstances, is certainly striking; is it, however, sufficient evidence to advance in the face of that of the proponents of the other theories, whose data are the result of observations on a variety of stains and technics?

If we put any faith whatever in our conceptions of tissue specificity, the idea that the pulmonary epithelium is alone capable of producing cells which, to all intents and purposes, not only resemble, but actually become indistinguishable from mesenchymal derivatives (when observed by a number of vital, supravital and fixed-tissue methods) is, to say the least, irrational. Were it established beyond a doubt that the epithelium could become transformed into connective tissue or adult mesenchyma — and *vice versa*, we might accept such an hypothesis with complacency.

Monocytic Origin

The interesting work of the Lewises ('23, to '25), Wislocki's ('24) findings and Maximow's ('26) long series of investigations, as well as the experiments detailed in this paper all point very strongly to the monocytic origin of the dust cell. Cunningham, Sabin and Doan distinguish between monocytes and clasmotocytes on a technical basis, depending upon the respective supravital staining characteristics. That this distinction is entirely warranted is still disputed by authors like Masugi, who either regard these types as phases of the same cell or take issue with the interpretation of their origin. Masugi considers that there are two types, monocytes ("Bluthistiozyten") and histiocytes proper; the Lewises regard these as different forms of the same cell; Maximow ('26) agrees with Masugi's interpretation in substance, but takes a broader view of the matter.

Mountain spotted fever (Wolbach, '19) and a number of other diseases, there is such manifest proliferation of the vascular endothelium that it might be construed as productive of epithelioid cells and phagocytes, but one could consider this change to be limited to the vessel wall and interpret the wandering phagocytes as emigrated monocytes, or histiocytes. Gardner ('26) points out an inherent weakness in the arguments of Permar and myself, when he says that we admit the ingestion of carbon by the monocytes and yet interpret them as being derived from the carbon-laden vascular endothelium. "But this method of demonstration," he continues, "is subject to the objection that within the vessels there are at least two types of cells which take up the vital stain — the endothelial lining cell and also the circulating white blood corpuscles." This is quite true; like McJunkin ('18, '19) I considered that the monocyte was produced by vascular endothelium, but the matter has taken a different turn as data have accumulated to give evidence to the contrary. Moreover, the vascular endothelium is a tissue that will bear further study, — it is not too well understood at the present time.

Lymphocytic Origin

The theory that pulmonary phagocytes, as "polyblasts," are derived from lymphocytes would, at first glance, seem to be susceptible to the same criticism as the epithelium or vascular endothelium. The lymphocytes do not stain in the same way, either vitally, supravitaly, or after silver impregnation, but Maximow's and Bloom's observations point too strongly to this possibility to permit its being lightly dismissed. We have no evidence that the lymphocytes become transformed into dust cells in the alveoli, but we cannot deny that they might become converted into monocytes in the circulation and thus enter the alveoli in a new guise and produce dust cells.

SUMMARY

The wealth of evidence adduced from the literature and the experiments here described go far toward proving that the most likely origin of the pulmonary dust cell is from the blood stream and, more specifically, the monocytes thereof. That various reticulo-endothelial elements, such as the supporting cells of the lymphoid tissue of the lung, the adventitial cells of its vessels or even lymphocytes

the observation of a transformation of lymphocytes in tissue cultures into polyblasts and fibroblasts, while alive and growing. His pupil, Bloom ('27), reports the transformation of lymphocytes, taken from the "water-clear" lymph of the thoracic duct of rabbits, into polyblasts. It is indeed difficult to remain skeptical in the face of this evidence, therefore we must add the circulating lymphocyte to the possible sources of monocytes and, through these, of dust cells.

Histiocytic Origin

That the histiocytes, or reticulo-endothelial cells of the pulmonary stroma share in the production of dust cells cannot be denied, but there is reason to believe that they play a subsidiary rôle. These cells do not become as sharply impregnated with silver tannate as do the dust cells, whereas the monocytes do. This does not prove that they are unassociated with dust cells, however, for they are in a different medium, more or less fixed and possibly correspond to the cells that spread out over the surface of the glass in tissue cultures or films. They were much increased in the experiments with milk injection and they contained much fat, hence they cannot be excluded as possible parents of dust cells.

Vascular Endothelium

That this tissue produces dust cells seems unlikely. The vascular endothelium does not stain in the same way and it appears to play an entirely passive part in these experiments. Where the reaction to the fat was most intense, the capillary walls seemed to be thickened, but as they were thronged with leucocytes and as the histiocytes and fibroblasts of the stroma were also more numerous, it was very difficult to ascribe to them any importance in the production of free phagocytes. While loth to retract my original claims concerning the rôle of the capillary endothelium in such conditions until convinced that they had become untenable, seven years of further investigation, a critical study of the experience of others, and the results of my recent study of these have all indicated that the time has come to admit that the origin of these phagocytes is more likely to be found in the blood, rather than in the capillary endothelium. In tuberculosis, measles (Mallory and Medlar, '20), typhus (Wolbach, Todd and Palfrey, '22), Rocky

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themselves, share to a degree in the production of dust cells cannot be denied; that they play the chief part in this production, however, seems improbable. That the vascular endothelium gives rise to dust cells under ordinary conditions seems entirely unlikely, in the face of evidence accumulated during the past decade. In view of this fact it will be necessary to readjust our theories of inflammation so that they may more nearly conform with those of Metschnikoff and the Lewises on the one hand and of Maximow on the other. This *volte face* on my part is made only after due deliberation and in the face of what seems to me to be overwhelming evidence.

CONCLUSIONS

1. The dust cells or "Herzfehlerzell" are probably larger forms of monocytes or blood histiocytes. While the tissue histiocytes may play some part in their production, it is more likely that their ranks are recruited from the circulating monocytes of the bloodstream. The origin of these is discussed.

2. The alveolar epithelium of the lung does not produce dust cells in so far as can be ascertained; it possesses totally different affinities for silver salts and can, by means of silver tannate impregnation, be readily recognized and differentiated from the alveolar macrophages, which appear to be of mesenchymal, rather than of endodermal origin. Furthermore, as has already been pointed out by other investigators, the reactions of these groups to supravital stains are equally divergent.

3. The assumption that there is no nucleated "respiratory epithelium" does not appear to be warranted, for sections supravitally stained with neutral red and counter-impregnated with silver tannate show pictures in every way similar to those drawn by anatomic investigators. As the refractive index of alveolar epithelium is very close to that of glass, it is imperative that some procedure be used that will render it artificially visible, or that the light in the condenser be arranged so as to utilize the very slightly higher refractive index of the epithelium, otherwise it will escape notice.

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DESCRIPTION OF PLATE

PLATE 120

- FIGS. 1, 2 and 3. These show cells supravitaly stained with Nile blue sulphate. Fig. 1 shows the rosette form of granular arrangement and in Fig. 3 (young dust cell) a neutral fat globule has displaced the Nile blue sulphate granules.
- FIG. 4. Monocyte stained with neutral red, supravital technic.
- FIG. 5. Monocyte stained with Sudan III and hematoxylin, after fixation in formalin.
- FIG. 6. Monocyte impregnated with silver tannate, after fixation.
- FIG. 7. A typical dust cell, stained supravitaly with Nile blue sulphate. It contains (a) Nile blue granules and vacuoles, (b) neutral fat droplets, (c) carbon particles and (d) refractile yellowish material. (Wislocki's "Type III Clasmatocyte".)
- FIG. 8. Young dust cell, impregnated with silver tannate after fixation. Compare with Fig. 3. A fat droplet is present, but has not displaced the granules which form a rosette.
- FIG. 9. A desquamated epithelial plate, supravitaly stained with Nile blue sulphate. Note the Y-shaped structures and the general pallor of the cell, also the nuclear remnant.
- FIG. 10. A bit of pulmonary capillary supravitaly stained with Nile blue sulphate and drawn on a smaller scale. The vascular endothelium is free from granules.

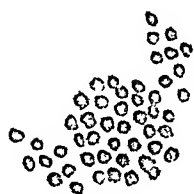
The cells were outlined with a camera lucida and drawn in freehand.

Figs. 1 to 6 inclusive are semidiagrammatic drawings of monocytes stained in various ways.

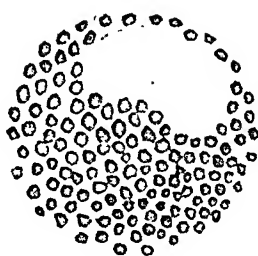
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PLATE 121

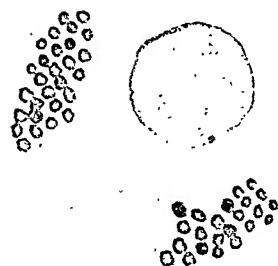
- FIG. 11. A monocyte in the blood of a pulmonary vein. Note the granules and the "attraction sphere" in the bight of the nucleus. Silver tannate technic. Oil-immersion photomicrograph, about $\times 1000$.
- FIG. 12. A group of monocytes in a capillary knot in a rabbit's lung. One of them has either emerged from the capillary, or is lying in an alveolar "niche." (Fenestrum?) Note the granules in these cells and the total absence thereof in the capillary endothelium. Silver tannate, oil-immersion. $\times 1000$.
- FIG.. 13. Dust cells in the alveoli of a rabbit's lung. Note the grouping of the granules and the absence of these in the alveolar epithelium and capillary endothelium. Some of the cells show fat vacuoles. Silver tannate. $\times 500$.



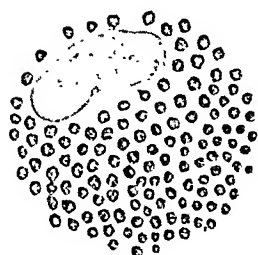
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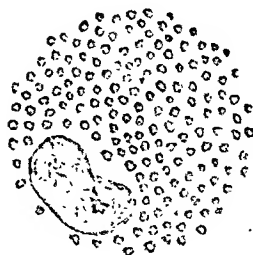
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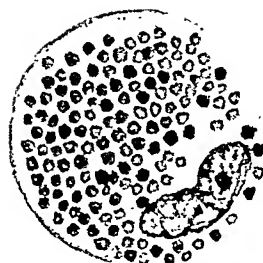
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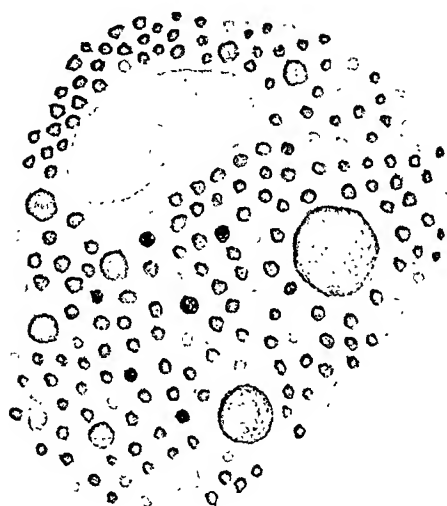
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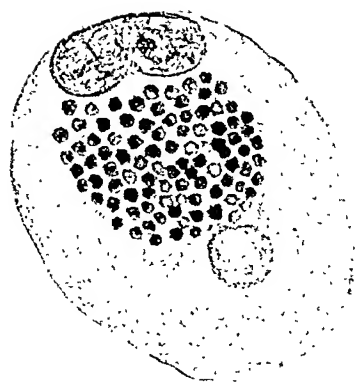
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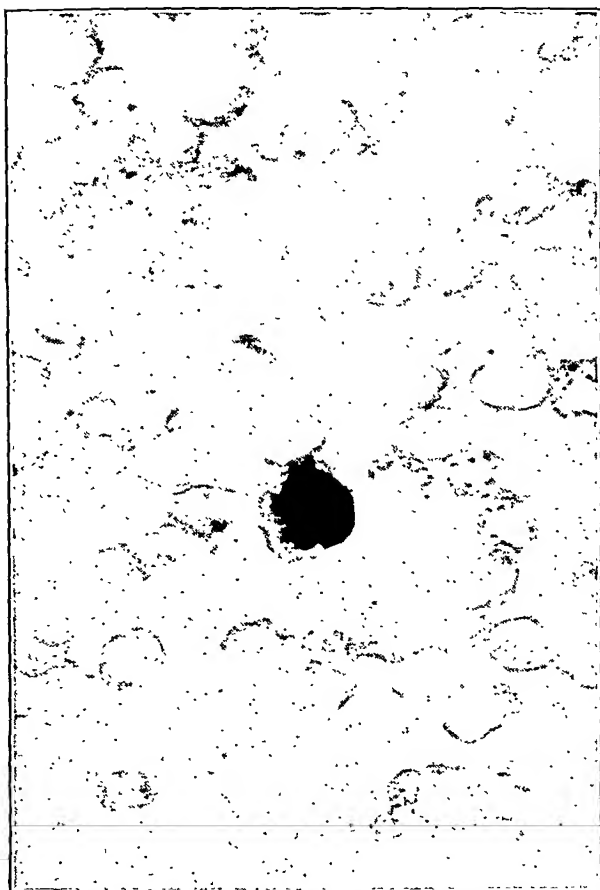
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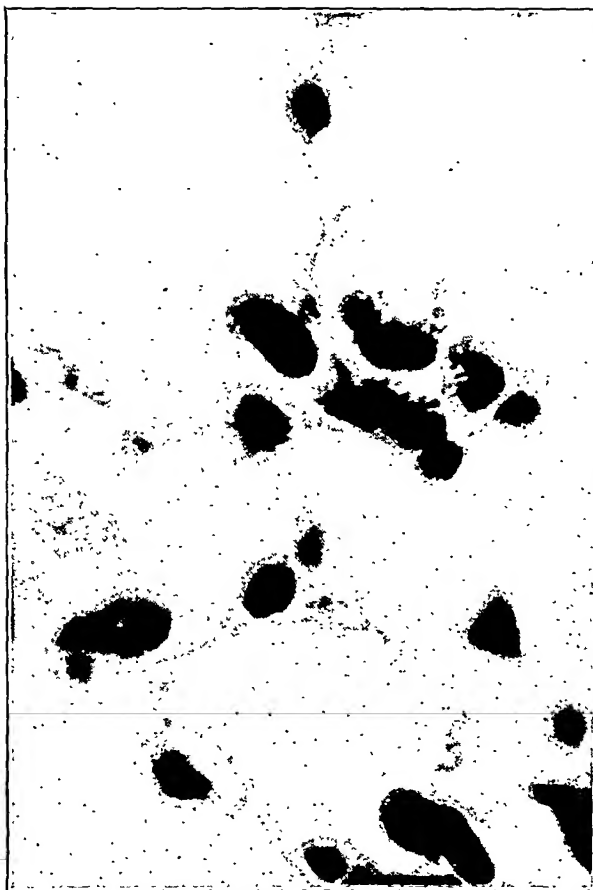
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PLATE 122

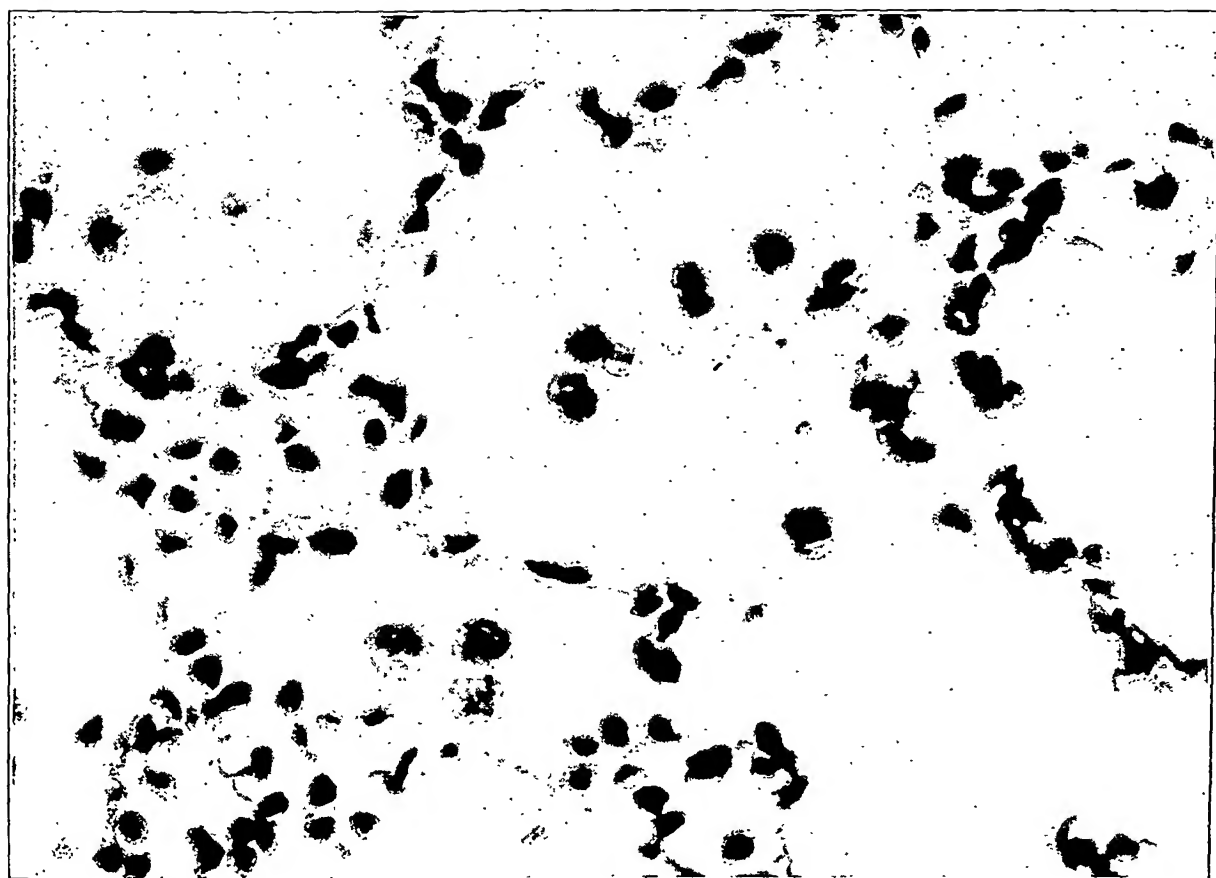
- FIG. 14. Surface view of alveolar epithelium. Note the occasional fenestrations, the interposition of dust cells in these and the outlines of the epithelial "flanges" (non-nucleated plates). *Cf.* with Ogawa's drawings. ('20). There are no sizable granules in the epithelium. Silver tannate. $\times 500$.
- FIG. 15. A thickened area in a rabbit's lung 24 hours after the injection of milk. This illustrates the difficulty experienced in identifying the component cells in such foci of inflammation. Silver tannate. $\times 500$.



11



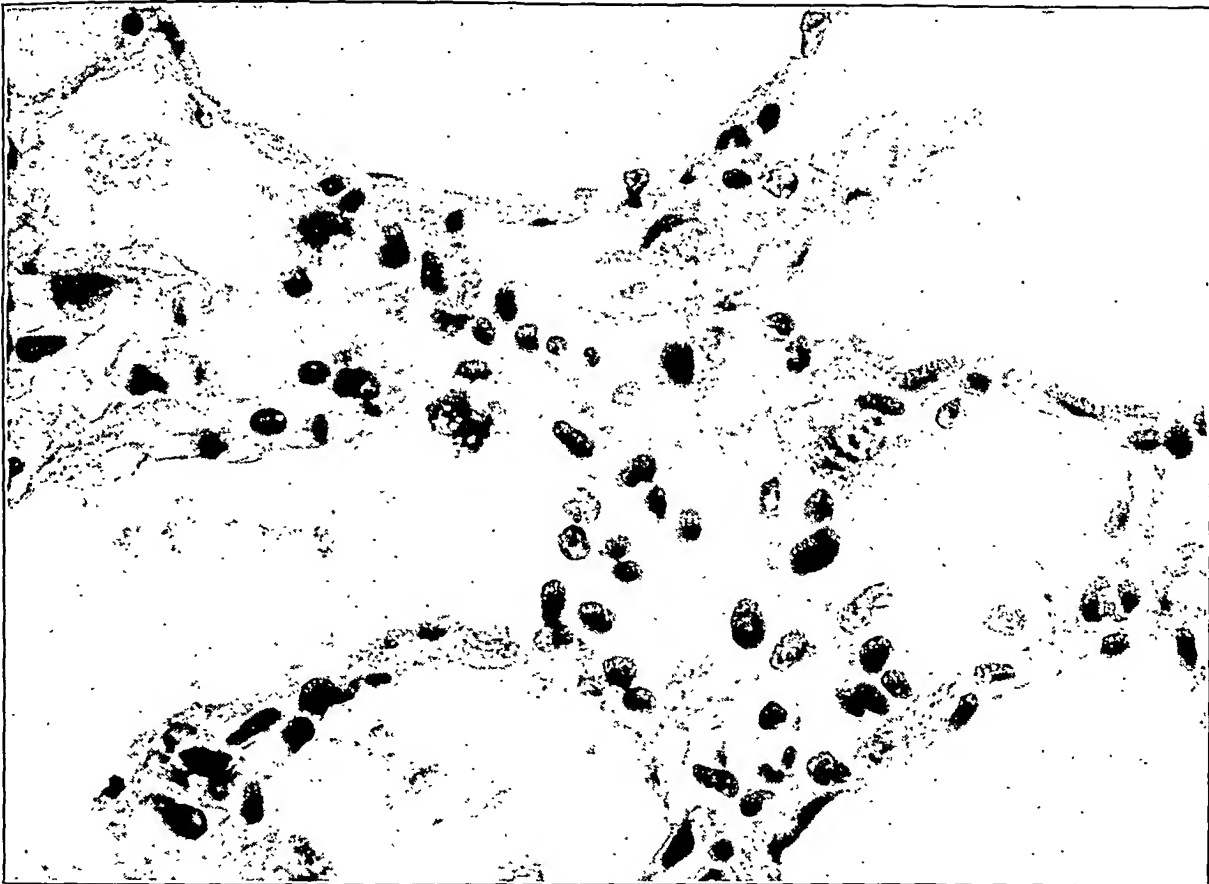
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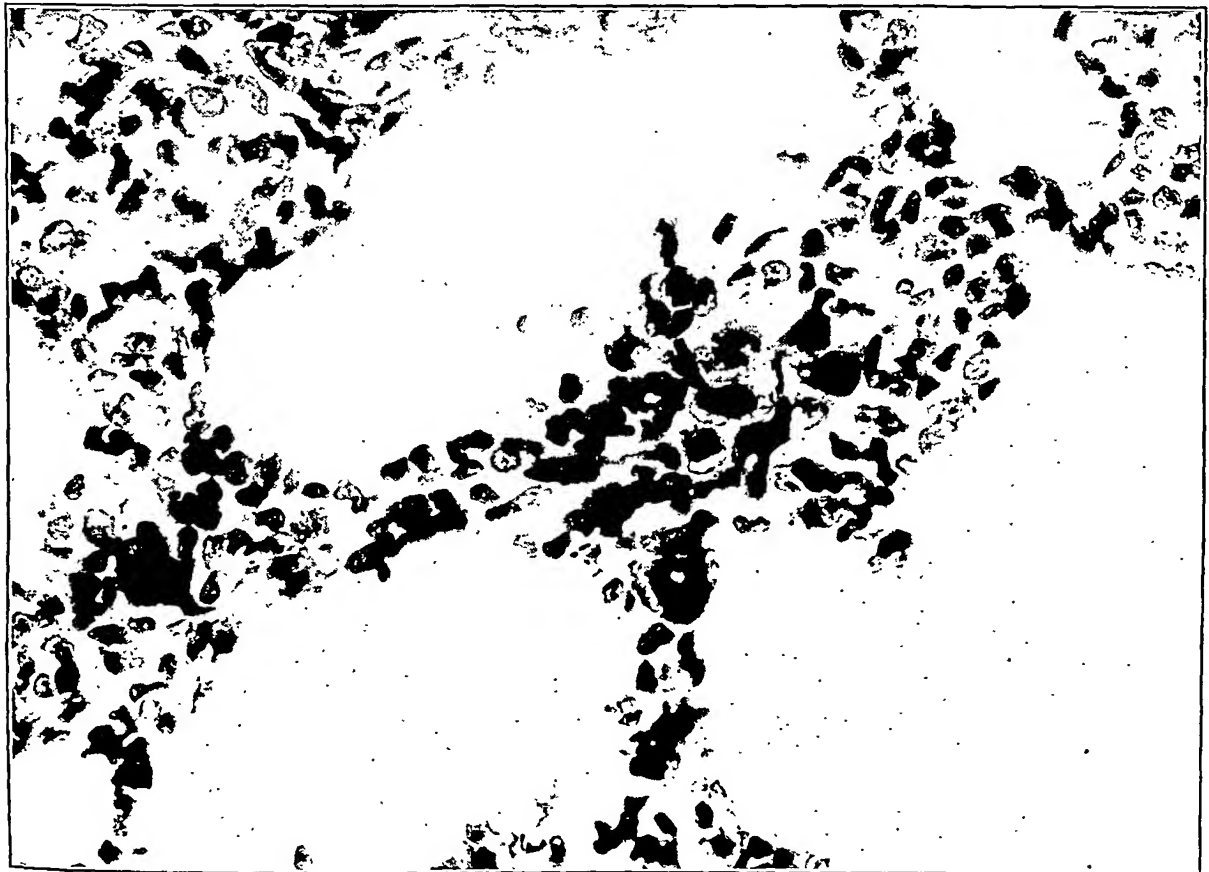
13

PLATE 123

- FIG. 16. Alveoli in a human case of chronic passive pulmonary congestion. Note the dense granules in the "Herzfehlerzellen," the comparative pallor of the desquamated epithelial cells, and the monocytes near the alveolar walls. The small, free granules between epithelial cells are coagulated albumen in edema fluid. Silver tannate. $\times 500$.
- FIG. 17. Hematoxylin and eosin section from the same block as that in Fig. 16. Note the absence of all granules other than hemosiderin or carbon and the misleading similarity in the appearance of desquamated epithelium and the dust cells, or "Herzfehlerzellen." Compare with Fig. 16. $\times 500$.



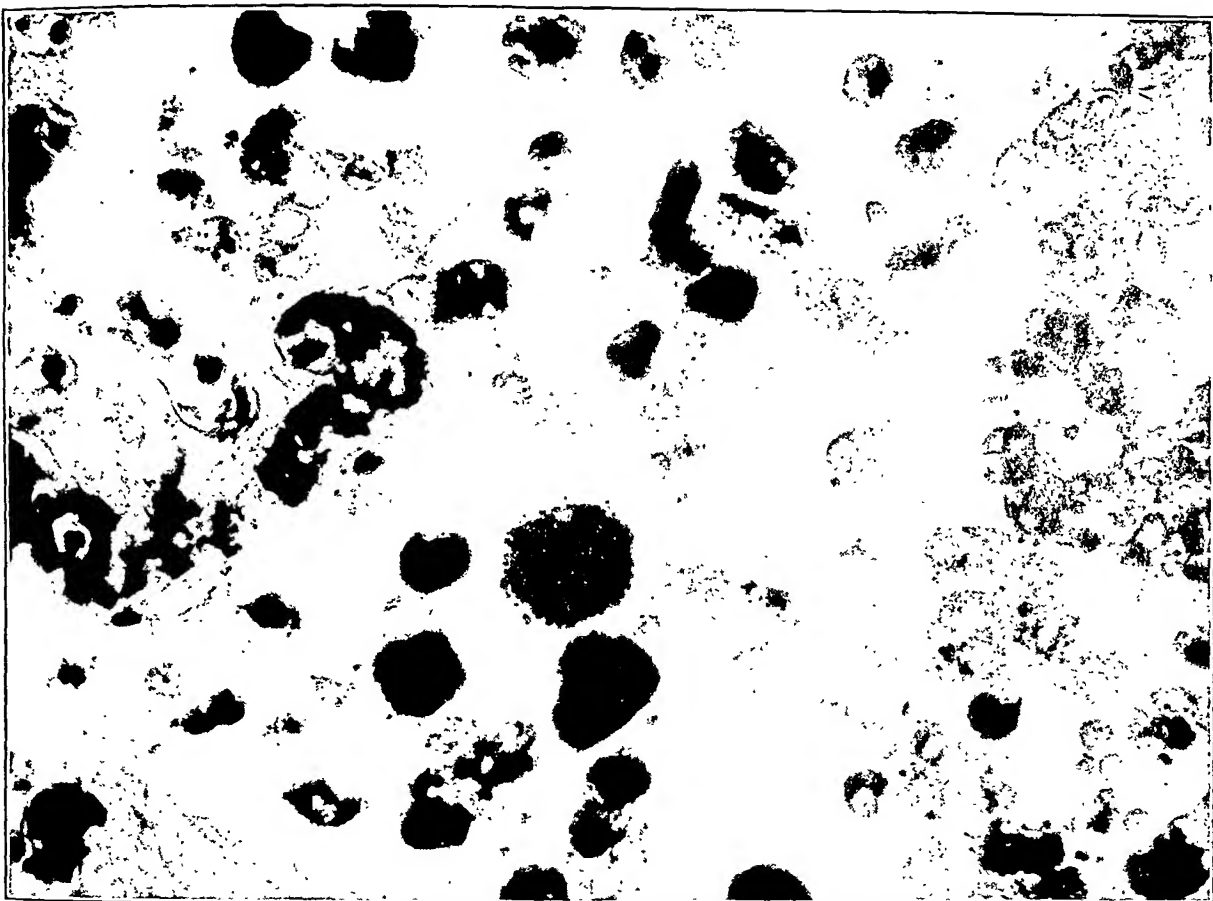
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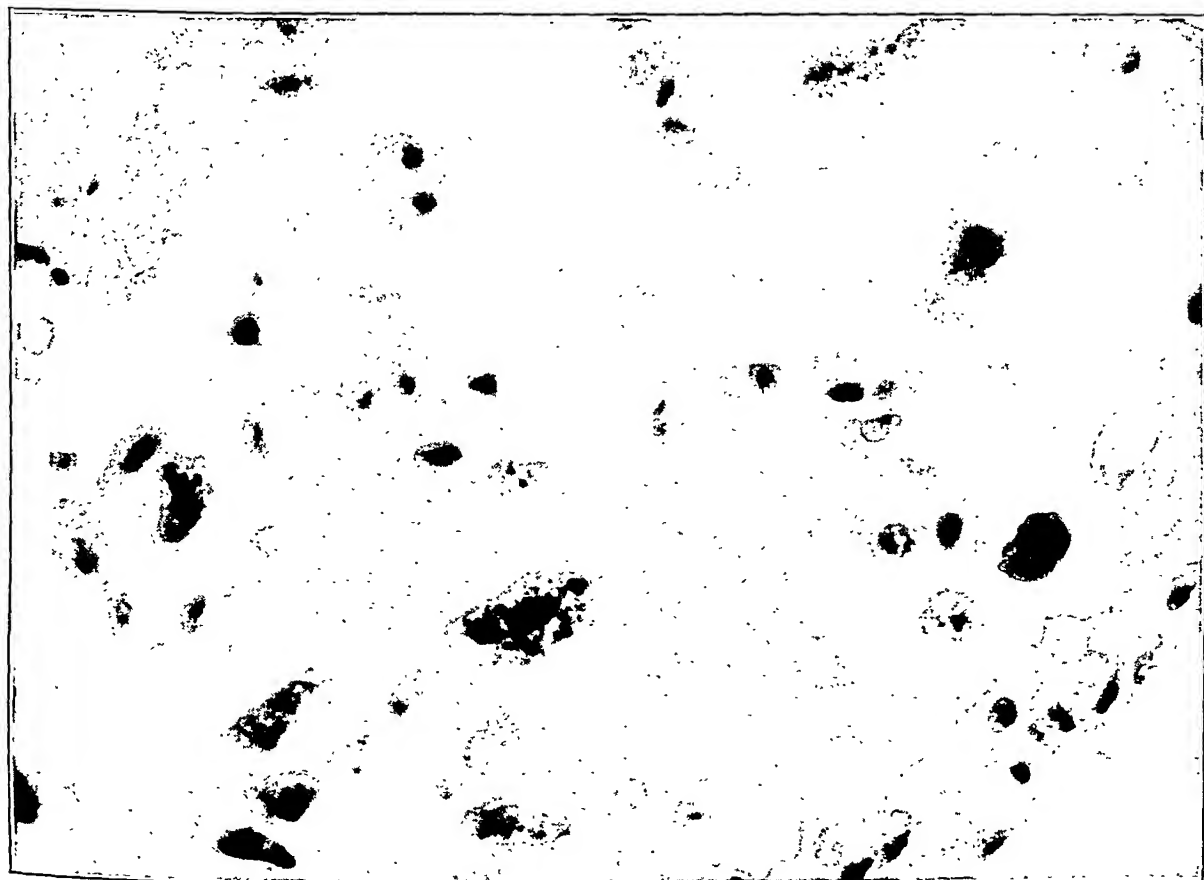
15

Foot

Origin of Pulmonary Dust Cell



16



17

Foot

Origin of Pulmonary Dust Cell

sues. Intravascular injections stain more especially the tissue and blood cells, whereas intra-alveolar cells often fail to assimilate the dye. The method which we have used is, in a sense, *supravital* in that the animal is killed by air embolism before injecting the dye. Staining is accomplished during a period of incubation of five to thirty minutes. While we were at work on the material to be reported, Cash² described *vital* staining with neutral red for the study of the histogenesis of the pulmonary tubercle. His method differs from ours in that he injected a smaller amount of concentrated dye into a vein ten minutes before killing the animal.

Our procedure has been as follows: In rabbits, 10 cc. of air is injected into an ear vein. In guinea pigs, which were most frequently used, the jugular veins are exposed under local anesthesia with novocain, and air is introduced. For intratracheal staining, the trachea is exposed in the neck, tied below the larynx and 30 to 60 cc. of a warm 1:1500* solution of neutral red in physiologic saline is injected into the lung. The whole animal is then incubated at 37° C. for a period varying from 5 to 30 minutes. The lungs are quickly removed and dropped into the fixative.

For intravascular staining, in most instances 50 to 80 cc. of the same dilute solution of dye is injected into a jugular vein; in some cases it has been injected into the carotid artery or the right ventricle of the heart. The dye may be introduced without first killing the animal by air embolism, but a guinea pig usually dies after the injection of more than 20 cc.

DESCRIPTION OF LUNGS STAINED BY NEUTRAL RED

Neutral red injected intravenously stains chiefly the cells of the normal lung structure; introduced by way of the trachea it not only stains the pulmonary cells, but it is sufficiently irritating, even in dilute solutions, to provoke the formation of a specifically stained mononuclear exudate in the air spaces. Under these conditions the whole process occurs after the death of the animal when the circulation has ceased. In some respects this stimulating capacity of dye is detrimental but we have turned it to advantage for the staining

* In our original description of the technic¹ we prescribed a dilution of 1:1500. With Grübler dye this is satisfactory. Subsequently we have used the product of The National Aniline Company and find that their more concentrated dye should be diluted 1:2000.

THE ORIGIN OF THE ALVEOLAR PHAGOCYTE STUDIED IN PARAFFIN SECTIONS OF TISSUE STAINED SUPRAVITALLY WITH NEUTRAL RED *

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from The New York State Hospital for Incipient Tuberculosis, Ray Brook, N. Y.)*

So much has been written on the origin of the alveolar phagocyte that it would be useless to offer further discussion of the problem, unless approached from an entirely new angle. The perfection of a technic for preserving supravital staining of the lung in paraffin sections has furnished such a new means of attack. The use of supravital staining with neutral red, which has accomplished so much in the reclassification of blood and exudate cells, has hitherto been limited to smears and scrapings. Whereas such preparations demonstrate the types of cells present they give no information as to the topographical relationship of these cells. However, when it becomes possible to study in thin paraffin sections the cells stained supravitaly as they appear in smears but preserved in their normal positions, a means is at hand to correlate the new cytologic data with the long established tissue morphology.

TECHNIC

The details of the technical procedures have been described elsewhere,¹ but it may be briefly stated that the cells of the pulmonary tissues in a living or freshly killed animal can be specifically stained by the intratracheal or intravenous injection of dilute solutions of neutral red in physiologic salt solution. Such staining can be preserved in sections embedded in paraffin by fixation in an alkaline Zenker-formaldehyde solution followed by rapid dehydration in mixtures of benzene and 95 per cent alcohol. The nuclei are counterstained with Harris' hematoxylin.

Both intratracheal and intravascular staining have been used because significant differences in effects have been observed. The former method stains all of the susceptible cells in the lung, those free in the air spaces and most of those in the blood and pulmonary tis-

* Received for publication June 29, 1927.

monary lymphoid tissues and to a much lesser extent in the alveolar septa. This is the typical clasmatoocyte, a large cell with a reniform or irregular nucleus surrounded by a cytoplasm which is packed with countless stained granules of variable size and shape (Fig. 1*d*). Sometimes nuclear fragments of phagocytosed cells can be distinguished among them. The pattern of these granules is not peculiar. In color they vary from light orange to deep maroon. The number of such cells in the lung can only be appreciated in vitally stained tissues, for they are overlooked in ordinary hematoxylin and eosin preparations.

A second type of cell which we have also classified as a clasmatoocyte is somewhat more conspicuous in sections stained in the usual manner. It is a large polygonal cell with vacuolated cytoplasm which projects from the surface of the alveolar septa. Sewell³ described it as being particularly abundant in the corners formed by contiguous septa. He suggested that it might be a specialized type of epithelial cell, a view which is held today by Aschoff and his pupils.⁴ Lang⁵ named it the "septal cell" and identified it with the histiocyte. We shall employ his nomenclature for purposes of discussion. All of these observers, irrespective of their views as to its origin, affirm that this cell is directly or indirectly the source of the alveolar phagocyte.

Sections of lungs stained supravitaly with neutral red bring out the great frequency of this cell in the septa. (Fig. 1*b*.) It projects into the lumen of the air space above and between the sparsely stained epithelial cells. Sometimes it assumes an hour-glass form which extends through the wall, the dilated terminal portions projecting into adjacent alveoli. (Fig. 2.) Neutral red is deposited in masses which occupy a large part of the cytoplasm. In size and color these masses are more uniform than in the typical clasmatoocyte, but a certain degree of variation exists. In lightly stained preparations the deposits assume the form of brilliantly refractile orange-red signet rings of variable size. (Fig. 1*b*.) If the staining time is increased their annular form is obscured and the deposits become solid masses, varying from orange to red. Phagocytosed nuclear fragments have never been observed in this form.*

* Because of the resemblance of the ring-shaped deposits of dye to small erythrocytes it has been suggested that they represent phagocytosed fragments of these cells, but in appropriate control preparations the rings fail to stain with eosin or to give a microchemical reaction for iron.

solution has served both as excitant and stain for the cells under investigation.

The reaction of the different types of cells to neutral red approximates that seen in smears and scrapings. However, fixation tends to neutralize the more delicate gradations of shade seen particularly in the clasmatocyte or macrophage. Furthermore it must be remembered that in sections the cells are cut at various angles, so that all planes cannot be brought into view as they can in smears. With these exceptions the cells in sections closely resemble those in smears.

A. INTRAVASCULAR INJECTIONS. When the stain is introduced into the vascular system it acts only as a dye and does not exert any appreciably irritating effect. When viewed with low power a section of a normal guinea pig's lung exhibits a surprisingly large number of brilliant, red granular cells situated in the connective tissues of the trunks and alveolar walls. The characteristics of the various supravitaly stained cells will be considered individually.

Epithelium. The columnar epithelium of the trachea and bronchi shows many large, deeply stained solid granules scattered at random throughout the cytoplasm. (Fig. 5.) The alveolar epithelium is best studied in thick sections (75 to 100 microns). These cells contain very few fine scattered cytoplasmic granules. (Fig. 1a.) In the ordinary thin sections (6 microns) these granules are occasionally found in the cut edges of the epithelium along the margins of the septa. The atypical vacuolated epithelium, which regenerates in chronic endemic pneumonia so common in guinea pigs, is apparently unable to retain the dye. In general, the arrangement of the stained cytoplasmic bodies of epithelium exhibits no characteristic pattern.

Endothelium. The cells lining the pulmonary blood and lymph vessels (Figs. 4a, 5c) do not stain specifically with neutral red, an observation which has been repeatedly verified in scrapings from the intima of larger vessels. In the specialized endothelia of the spleen, lymph nodes and liver (reticulo-endothelium and Kupffer cells) neutral red staining is very abundant but this does not concern us here.

Mesothelium. The cells covering the visceral pleura exhibit occasional fine cytoplasmic stained granules.

Clasmatocytes. (Histiocytes or macrophages.) Two types of this cell are found in the lung. The first regularly occurs in the connective tissues about the larger bronchi and vessels, in the intrapul-

intravascular injections of the dye. Only the effects upon the septal cells and the free phagocytes need further discussion.

Septal Cells. After an incubation period of five minutes following intratracheal staining the septal cells in no way differ from those stained intravenously. But thereafter many of them begin to lose their capacity to react supravitaly. After ten minutes' exposure the previously annular deposits become solid and are much reduced in number. The cell now shows only a few large scattered granules in place of the closely packed ringlets which formerly filled the entire cytoplasmic substance. After thirty minutes there are practically no granules and the nucleus is tinged with red. One hour after staining the cytoplasm is filled with colorless vacuoles and even the nucleus has lost all traces of neutral red.

A much more significant reaction to intratracheal injections of neutral red is a marked diminution in the number of septal cells after prolonged irritation. Many sections from animals incubated from thirty minutes to one hour or longer show septum after septum without a single one of these cells. The diminution in the number of cells is not merely an apparent one because of their failure to stain, for the degenerative changes just described are always sufficiently definite to mark them if they are present. We believe that a desquamation of septal cells has occurred provoked by the presence of an irritating solution in the air spaces.

Free Alveolar Phagocytes. As early as five minutes after intratracheal injection of a dilute solution of neutral red the air spaces contain many large cells, each of which is characterized by the presence of a compact rosette of brilliant orange granules situated in the "hof" of an indented nucleus. The cells and the components of the rosette are much larger than those of the typical monocyte. In ten minutes the number of these free cells has greatly increased, and there are some with two or three nuclei. By thirty minutes they have become still more numerous and giant cells with as many as twelve or fourteen nuclei can be found. After an incubation period of one hour most of the dye has disappeared. A few of the free alveolar cells still retain large scattered granules and very rarely the remains of a rosette can be detected. Occasionally typical monocytes are encountered but these are rare except in the earliest periods. Polymorphonuclear leucocytes and lymphocytes are practically absent.

The septal cell is peculiarly susceptible to overstaining with neutral red. With excessive exposures the stainable substance appears to be dissolved so that only its outlines are left, producing the vacuolization regularly seen in sections prepared by ordinary technics.

Tissue Eosinophiles. In the guinea pig's lung the connective tissues contain numbers of deeply stained, finely granular cells, often with elongated cytoplasmic processes. On closer examination it is seen that these granules are uniformly small and of a deep brick-red color. They stain more readily and resist decolorization more tenaciously than do the granules in any other type of cell. Their nuclei are bilobate or polymorphous.

By one accustomed to studying preparations of blood and exudates supravitaly stained with neutral red, these cells would unquestionably be classified as mast cells because of the color of their granules. However, control sections of the same material, stained with hematoxylin and eosin or with eosin and methylene blue, exhibit correspondingly large numbers of typical eosinophiles in the same locations. Furthermore in lungs of rabbits, an animal notably deficient in true eosinophiles, this deeply stained, finely granular cell is extremely rare. For these reasons we are compelled to classify this cell as an eosinophile.

Blood Cells. Both within the vessels and after migration into the tissues the cells of hematogenous origin exhibit typical staining. The monocyte is characterized by the familiar rosette of fine granules situated in the "hof" of the nucleus. Lymphocytes show a few scattered neutral red granules if the plane of section happens to include them. Often, however, this is not the case and none is seen. The Kurloff bodies, which we believe occur in lymphocytes, are deeply stained. Polymorphonuclear neutrophils are usually lighter and more yellow in color than those seen in smear preparations.

Fibroblasts. With prolonged exposure to the dye most of the fibroblasts contain occasional scattered pale orange to pink granules. Otherwise their appearance is not characteristic.

B. INTRATRACHEAL INJECTIONS. When introduced through the trachea, dilute solutions of neutral red not only stain the cells in the fixed tissues and blood stream, but provoke the migration of many beautifully stained phagocytes into the air spaces. In general the staining of the normal cells in the lung is the same as that seen after

the alveolar septa. Monocytes are less numerous in these recently killed animals, than in those to be described later where the dye is injected into the lungs during life.

SUPPLEMENTARY OBSERVATIONS

So astonishing was the very rapid accumulation of large rosette cells within the air spaces in the short period of five minutes that it seemed necessary to be certain that none was present at the time when the dye was injected. Accordingly a series of one-day old guinea pigs, in whose lungs the absence of dust precludes the presence of many phagocytes (Tchistovitch ⁷), were stained in the same manner. In their lungs also the same rapid accumulation of large rosette cells followed the injection of the dye.

To study the reaction of more mature alveolar phagocytes to neutral red, the lungs of animals exposed for a year or more to the inhalation of coal, carborundum, quartz and granite dusts were stained intratracheally. These lungs were known to contain great numbers of dust-filled phagocytes which had remained in the lung for a prolonged period. In their lungs the dye provoked a fresh accumulation of large rosette cells but the dust particles were contained in older cells exhibiting not a rosette arrangement but the diffusely scattered staining characteristic of a clasmatoocyte. Only rarely a fragment of dust was found in a cell with the rosette grouping.

To ascertain whether the reaction in the animal recently killed was the same as that in the living guinea pig, the following experiment was performed: A group of four pigs were anesthetized by intraperitoneal injection of amytal. Holding them in an upright position 3 to 4 cc. of neutral red solution was allowed to drop into their nostrils. Subsequent necropsies showed that the major portion of the dye was aspirated into the lungs. They were killed by air embolus at intervals of thirty minutes to three hours afterwards. The right pulmonary artery was clamped, and about 30 cc. of dilute neutral red was injected into the right ventricle of the heart. This treatment restrained the cells of the left lung, while those of the right lung exhibited only the effect of the primary intratracheal staining.

Study of the sections from the right lungs, stained only intratracheally during life, demonstrates the fact that within thirty minutes after instillation, the neutral red taken up by the majority of the cells is decolorized and reduced to a greenish black pigment.

The rosette cell which suddenly appears in the air spaces does not resemble a monocyte. It is nearly twice the size of that cell and, with the lapse of time after staining, the dye granules constituting its rosette begin to exhibit variation in shape, size and color. At five minutes they are ring-shaped like those of the septal cells, but later they become solid masses of color which varies from pale orange to deep maroon. The rosette formation is preserved for at least thirty minutes, but at that time a few granules of dye appear at the opposite side of the nucleus. Still later the dye appears to have been dissolved and excreted; the majority of the cells then stain only with hematoxylin. They are much more resistant to the toxic action of the neutral red than the septal cells. Nuclear staining is rarely seen.

During the period of thirty minutes after their appearance, the large rosette cells only occasionally become phagocytic. Even in the presence of a great abundance of available material they usually fail to assume this function. In the earlier stages of the work the animals were killed by section of the cervical vessels, and much blood was aspirated, yet these large rosette cells showed no ingested red corpuscles. Rarely, however, one does contain a carbon particle or two.

The multinucleated cells seem to be formed by fusion, but purely morphologic evidence is deceptive. They exhibit a peripheral ring of nuclei surrounding a very large central rosette. In some of the older preparations this rosette resembles that described by Cunningham, Sabin, Sugiyama and Kindwall ⁶ as characteristic of the epithelioid cell, an increase in the amount of stainable substance with a great multiplication of very fine particles at the center of the rosette and a segregation of the larger ones at the periphery.

Intratracheally, neutral red stains the alveolar epithelium in the same manner as intravascular injections but it causes no desquamation of these cells. In thick preparations it is possible to find areas in which the plane of section includes sheets of delicate nucleated cells covering the entire surface of an alveolus. These cells retain their original position after prolonged irritation and do not migrate like the septal cells.

As the duration of the irritation becomes greater, increasing numbers of typical small monocytes are found in the connective tissues, particularly about the vessels and bronchi. A few can be found in

ternal jugular vein. The perfusion was continued about five minutes. Clear fluid returned through the incised aorta and the lungs were apparently bloodless. Neutral red was then introduced through the trachea in the usual manner and the bodies were incubated twenty to thirty minutes.

Sections of these lungs demonstrated an almost complete removal of blood cells from the vessels. Alveolar phagocytes are formed in the same abundance as seen in the non-perfused animal. However, these cells do not exhibit the large rosette which was so characteristic in the former group. The neutral red granules are scattered diffusely throughout the cytoplasm, an arrangement more suggestive of the clasmatocyte type. The cells appear much swollen, a manifestation of the generalized edema produced by perfusion. Monocytes are extremely rare but in the connective tissues occasional unstained cells are encountered whose general morphology is suggestive of this type.

To determine whether capillary endothelium may be so altered during intrapulmonary irritation that it acquires the property of reacting to neutral red, the following experiments were tried: Without anesthesia, 2 cc. of filtered India ink, diluted 1 to 4 with distilled water, were allowed to run into the nostrils of 10 guinea pigs. They were killed by air embolism at intervals from 30 minutes to 4 days. All but one animal showed gross pigmentation of the lungs with ink. The lungs were stained by the intratracheal injections of neutral red.

In no instance is it possible to demonstrate specific staining of vascular endothelium and no evidence of division of this cell can be detected. The alveoli contain tremendous numbers of phagocytes exhibiting the diffuse clasmatocyte grouping of neutral red and the septa are crowded with similar cells. In both locations the majority of the cells contain ink.

In six other animals an edema of the lung was produced by a two minute exposure to chlorine vapor as reported by Haythorn.⁸ They died or were killed in thirty minutes to one hour. Subsequent intratracheal injection of neutral red demonstrated that the majority of the pulmonary cells were so injured by this treatment that they either completely failed to stain specifically or the staining was very weak. No significant changes in the vascular endothelium were observed.

Only the eosinophiles contain unchanged dye. The irritation in the living animal with an active circulation provokes an exudation not only of large mononuclear phagocytes, but of a few polymorphonuclear neutrophils. The presence of stained eosinophiles, of exudation and of changed pigment is conclusive evidence that the dye successfully penetrated the lung of the living animal.

Sections of the opposite lungs, retained through the blood vessels, demonstrate the fact that the cells containing the decolorized dye do not lose their capacity to react. Free phagocytes containing the older greenish black particles of the intratracheal neutral red again take up the fresh stain which is deposited in diffusely scattered granules and vacuoles having a characteristic clasmatocyte grouping. In many instances the new dye is superimposed upon the old. Some of the cells contain phagocytosed cellular debris. The color of the stained elements varies from light orange-pink to deep maroon. Giant cells are rare but they do occur, particularly in the animal killed at thirty minutes.

The septal cells also exhibit double staining and many of them now react like clasmatocytes. Evidence of diminution in the number of these cells is hard to demonstrate in the living animal, but the impression is gained that shortly after irritation the septa surrounding air spaces which contain many free phagocytes show fewer septal cells than those in other areas. Three hours after intranasal treatment the septa seem to contain even more than the normal number of these cells.

Typical small monocytes appear in large numbers in the connective tissue at thirty minutes after intranasal staining and in the alveoli after three hours.

An elimination of injured clasmatocytes from the lung is suggested by the appearance of partially stained cells of this type in the lymphatics. Their number steadily increases from one to three hours after intrapulmonary irritation.

To ascertain whether the cells of the blood retained in the pulmonary vessels after the circulation has ceased, play a part in the formation of alveolar phagocytes, lungs perfused with Ringer's solution were stained intratracheally. Six guinea pigs were anesthetized with amytal. The abdominal aorta was cut, a clamp was placed upon the inferior vena cava and 50 cc. of warm Ringer's solution containing heparin, one part in 10,000 was injected through an in-

sembles that of the free phagocyte. It is difficult to believe that within five minutes time the sparsely scattered fine granulations of the epithelial cell could be transformed into the rosette of large densely stained rings or globules of dye seen in the free phagocyte. Furthermore the demonstration in the thick sections of an intact sheet of lining epithelium without evidence of desquamation is opposed to the view that the phagocyte is derived from this source. The contention of Aschoff and his pupils⁵ that the septal cell is a special type of epithelium will be discussed later.

Vascular endothelium as a source of alveolar phagocytes has been extensively investigated by Haythorn^{8,9} Permar^{10,11} and Foot.¹² The evidence derived from irritation and supravital staining with neutral red offers no support to this origin. The vascular endothelium of the lung does not react specifically to this dye. The only evidence of staining has been in the nuclei of dying cells. The technique employed is adequate to demonstrate the presence of mitosis or indirect division. Prolonged search has failed to demonstrate a single example of proliferation in the local capillary endothelium. Even after preliminary irritation with India ink these cells have failed to exhibit evidence of multiplication or of specific staining. It must be admitted that we relinquish this origin with considerable reluctance for previously we ourselves were convinced that in general vascular endothelial cells were the source of such phagocytes. However, the data submitted prohibit this conclusion.

There remains only the clasmatocyte or histiocyte group of cells, in which we have included the septal cells of the alveolar walls. We agree with Lang¹⁴ that septal cells are somewhat modified clasmatocytes, but our judgment is based on their reaction to neutral red in supravital staining. Both forms exhibit a cytoplasm filled with specifically stained granules which in light exposures assume the form of refractile signet rings, but as the staining time increases they become dense masses which vary in depth of color. The following differences exist. The clasmatocyte is regularly phagocytic if the proper material is available; the septal cell is only rarely so unless the alveoli contain excessive amounts of foreign substance. The nucleus of the clasmatocyte may be reniform or irregular while in the septal cell it is generally round. This variation in function and morphology might conceivably be due to differences in position.

On the other hand, Aschoff holds that the septal cell is modified

DISCUSSION

Alveolar phagocytes are not present in any great number in the normal lung, but they accumulate rapidly after intrapulmonary irritation. The rapidity with which they accumulate in the air spaces suggests that they arise from some source readily available. Three possibilities are conceivable: (1) Multiplication of preëxisting phagocytes; (2) migration of cells from the blood stream; (3) simple migration or multiplication followed by migration of some cell in the permanent structure of the lung. The first source, preëxisting free cells, is eliminated because it was demonstrated that free phagocytes accumulate with the same rapidity in the air spaces of very young animals which, previous to irritation, contain no cells of this type. Even though there were preëxisting intra-alveolar cells, five minutes is too short a period to permit cell division of this magnitude to occur. The second source, the blood stream, is eliminated because alveolar phagocytes accumulate after the death of the animal, when the blood is no longer circulating. A possible origin from the blood cells retained in the pulmonary vessels has been eliminated in the experiment where the blood was thoroughly washed out of the vessels previous to intra-alveolar irritation and staining.

Even though the circulation was intact, this cell could probably be eliminated, for Smith, Dworski and Gardner¹³ have reported a series of cell counts, made of blood from the right and left ventricles of the heart at intervals after inhalation infection with tubercle bacilli, in which the number of monocytes in the blood entering the lungs did not sufficiently exceed that in the effluent blood to account for the great number of epithelioid cells in the lung. We therefore eliminate monocytes from the blood as direct ancestors of the alveolar phagocyte. For these reasons discussion is limited to a consideration of some cell in the essential structure of the lung.

The local origins which have been proposed for the alveolar phagocyte are as follows: Alveolar epithelium, vascular endothelium and connective tissue clasmatoocytes or histiocytes which include the septal cells of Lang. It becomes necessary to examine each of these cells and to discuss the claims made for them in the light of the evidence previously presented.

The staining of the general alveolar epithelium in no way re-

a condition which has been interpreted as a manifestation of regeneration.

The source from which regeneration of septal cells proceeds cannot be absolutely proved. Mitotic figures or evidence of indirect cell division is wanting in any cell in the vicinity. However, it is noteworthy that within ten minutes after introducing the irritating dye into the air spaces large numbers of typical small monocytes, not previously encountered, become visible in the pulmonary connective tissues. Cunningham, Sabin and Doan¹⁵ are convinced that such cells develop from indifferent reticulum cells in various parts of the body. Lacking any other origin it has occurred to us that these small monocytes may undergo a process of maturation and evolve as connective tissue phagocytes and septal cells. They appear on stimulation at the same time when the septal cells are desquamating and they again decrease in number and finally disappear after the climax of the reaction. A few leave the connective tissues and get into the air spaces, but the majority of them remain inside the framework of the lung.

SUMMARY

The stages of the histogenesis of alveolar phagocytes as we have observed them may be summarized as follows: Under stimulation the septal cells are shed into the alveoli and are transformed into free phagocytes. New septal cells are formed from connective tissue monocytes which in turn arise from the indifferent reticulum cells.

In formulating this hypothesis we have not insisted that the neutral red stained masses must always retain the same pattern regardless of the position and physiologic state of the cell. The reticulum cell shows no staining, the tissue monocyte exhibits a small rosette in the "hof" of an indented nucleus, the clasmatoocyte has diffusely scattered granules of various sizes and colors, while the free phagocyte displays different groupings which vary with its age, at first a rosette and later a diffuse distribution. This variation can be proved by observation in the last named cell, a fact which lends plausibility to the concept as a whole. Cytoplasm is labile and rearrangement in its structure must occur with changes in mechanical conditions and the assumption of new functions.

In arriving at this conclusion we believe that we have offered further evidence to substantiate the observations of Lewis¹⁶ that

epithelium but in supravitality stained preparations it bears no resemblance to any other epithelial cell in the body, and much less to those in the alveoli and bronchi. The general lining cells of the alveoli show widely scattered very fine droplets of stain. Between these, along the connective tissue septa, the septal cell with its heavy masses of deeply stained granules stands out in sharp contrast. Furthermore, it has been shown that epithelial cells do not desquamate after irritation whereas septal cells do.

The supravital reactions of the septal cell to neutral red so closely resemble those of clasmatoocytes in general that we have classified it in this group in spite of certain morphologic and physiologic differences. In this conclusion we have concurred with Lang¹⁴ whose inferences were drawn from a study of tissue cultures.

The similarity between septal cells and free alveolar phagocytes is equally striking in supravitality stained tissues. Both present annular refractile globules after short exposures to the dye which later become dense and homogeneous and both show well defined variations in the color of their granules. On the other hand there are differences; the sessile cell exhibits diffusely scattered cytoplasmic granules while in the free cell, when it first appears in the air space, these granules are closely packed into a rosette situated in the "hof" of an indented nucleus. With age they become scattered and the rosette is lost. The stainable substance in the septal cell is destroyed or dissolved after prolonged exposure to the dye but the cell can recover so that the same elements will be restrained. In the free cell the combination of the dye with the cytoplasmic elements is more resistant and is not destroyed so quickly. The free cell is phagocytic; the sessile cell usually is not.

We believe that under the stimulation of foreign substances introduced into the alveoli, the septal cells desquamate and are transformed into alveolar phagocytes. It is difficult to prove with certainty that the number of sessile cells is actually decreased because all of them are not shed at one time but the impression is strong that they are diminished five or ten minutes after intratracheal injection of neutral red. It is more obvious in the animal recently killed than in the living one, because in the latter regeneration commences at once and replacement of the desquamated cells is constantly taking place. In the living animal, three hours after intranasal instillation of the dye the number of the septal cells is very obviously increased,

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DESCRIPTION OF PLATE *

PLATE 124

- FIG. 1. (a) Alveolar epithelium; (b) Septal Call; (c) Free Phagocyte, "large rosette type"; (d) Free phagocyte, older form with clasmatoctye grouping of neutral red; (e) Polymorphonuclear neutrophile, degenerated form; (f) Free phagocyte, monocyte-like form; (g) Eosinophile.
- FIG. 2. Septal cell projecting into adjacent alveoli.
- FIG. 3. Fibroblast.
- FIG. 4. Giant alveolar phagocyte; (a) and (a) capillary endothelium.
- FIG. 5. Bronchial epithelium; (a) Eosinophile with elongated processes; (b) Polymorphonuclear neutrophile; (c) Capillary endothelium.
- FIG. 6. Polymorphonuclear neutrophile.
- FIG. 7. Blood vessel. Endothelium and smooth muscle show no specific stain; (a) Polymorphonuclear neutrophile; (b) Eosinophile; (c) Monocyte.
- FIG. 8. Mitotic figure in a cell applied to the surface of an alveolar septum.

* All drawings made from preparations of animals stained intratracheally.

alveolar phagocytes arise from monocytes. In the living frog she was able to observe a direct transformation of the cell in the blood into the pulmonary phagocyte, and she has also observed corresponding morphologic changes in hanging-drop preparations of blood.¹⁶ In recently killed guinea pigs where the circulation is no longer functioning we have seen evidence that extravascular monocytes, probably produced locally in the connective tissues, gave rise to clasmatocytes and septal cells which in turn migrated into the air spaces and were there able to assume the function of phagocytosis. Our interpretation of this evidence has failed to substantiate the view of Sabin and her co-workers that the monocyte and clasmatocyte, as differentiated by supravital staining with neutral red, are two separate and distinct types of cells. We believe that the grouping of neutral red-stained elements in a cell can vary with its position and physiologic state.

CONCLUSIONS

The data obtained from a study of the lungs of guinea pigs stained supravitaly with neutral red, immediately after death by air embolism, have suggested the following deductions:

1. The alveolar phagocyte is produced by the desquamation of septal cells.
2. The septal cell belongs to the general group of connective tissue phagocytes or clasmatocytes.
3. These clasmatocytes appear to arise from previously inactive reticulum cells which pass through a monocyte-like phase in the process of maturation.
4. Alveolar epithelium and local vascular endothelium play no part in the origin of the alveolar phagocyte.
5. The arrangement of the neutral red stained cytoplasmic elements is not fixed and cannot therefore be used as a criterion to differentiate clasmatocytes from monocytes.

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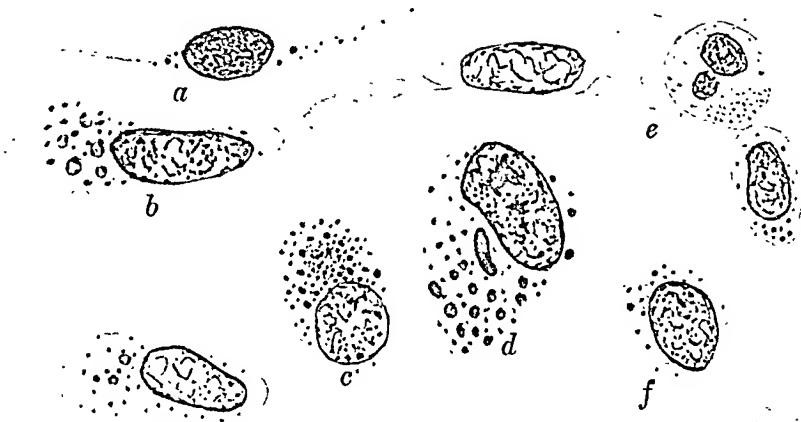


Fig. 1

Fig. 2

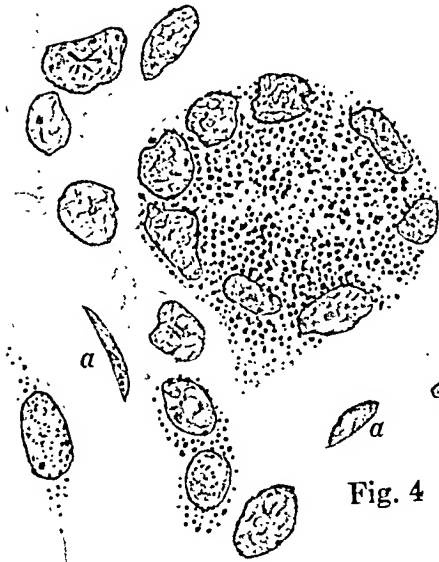


Fig. 4

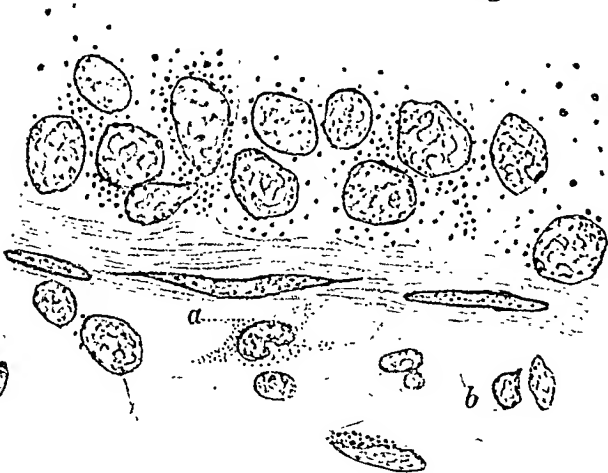


Fig. 5

Fig. 3



Fig. 6

Fig. 7



Fig. 8

and which separated the parenchymal cells from the neighboring vessels. The islet cells were atrophic or absent, and when the process had progressed, the whole structure was replaced by hyalin. In many places, so advanced was the destructive change, the hyaline substance was found to extend beyond the limits of the islands into the surrounding interacinar connective tissue. The primary lesion, however, was quite evidently within the insular spaces.

In a subsequent paper, Opie³ reported another case in which hyalin was found, this time definitely confined to the Langerhans areas. The degree of involvement varied. Rare islets appeared to be uninjured. In others there were only a few small scattered masses of hyalin here and there in the island, closely associated with the capillary walls. Where the lesion was most advanced the island was practically replaced by the hyaline, homogeneous material in which a few elongated, compressed, pycnotic nuclei of parenchymal or endothelial cells remained. Often the parenchymal cells had entirely disappeared.

Following Opie's reports in which this peculiar hyaline degeneration of the islets was first described, there appeared other descriptions of a similar change, likewise limited to the insular tissue, in diabetic pancreases, Wright and Joslin,⁴ Schmidt,⁵ Herzog,⁶ Müller,⁷ Norris,⁸ Cecil⁹ and others. In every way the hyaline material was similar in nature and distribution to that described by Opie.

In all of these reported cases showing hyaline degeneration of the islets, diabetes mellitus was present. Not only was the disease itself evident, but it had usually existed for a long period, at least one year, and often three or more. The average duration in sixteen of the twenty-seven cases cited by Cecil was three years and six months. Furthermore, the lesion rarely occurs in patients under forty-five years of age. Of twenty-seven cases, Cecil found six who were under forty-five, while Warren and Root¹⁰ report a single patient out of thirteen who was under forty-five. Cecil's youngest was a boy of twelve.

The lesion, then, in about seventy-five to eighty per cent of the cases is present in patients over forty-five; and this type of degeneration thus characterizes a certain type of diabetes which is most commonly found after middle life. It may be present with or without a coexistent interacinar sclerosis, but is rarely found associated with an acute process. The insular changes which are found in younger

HYALINE DEGENERATION OF THE ISLANDS OF LANGERHANS IN NON-DIABETICS *

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Abundant evidence is at hand today to prove a causal relationship between degenerative or destructive lesions of the islands of Langerhans and the metabolic disturbance, diabetes mellitus. Initiated by the experiments of von Mering and Minkowski¹ in 1889, in which glycosuria was found to follow total extirpation of the pancreas in dogs, the relationship of the pancreas to this disease has been the subject of many investigations. In general these studies have followed one of two courses: first, the strictly experimental, in which a study of pancreatic physiology, both normal and abnormal, has been carried out through controlled animal experimentation; and second, the purely descriptive, in which the pancreas of the diabetic human has been made the subject of thorough morphologic study. In this second field little advance was made until about 1900 when Opie, in a series of papers, reported the results of his careful observations. Up to this time the pancreas was not thoroughly and systematically studied with the microscope, and most of the descriptive pancreatic pathology was based on macroscopic findings. It was Opie who described for the first time the most important histologic changes in the diabetic pancreas, and who called attention to the frequency with which the islands of Langerhans were involved. To one of the insular lesions, a degenerative process associated with the presence of a peculiar hyaline substance, described first in 1901, I wish to call special attention.

In his original paper, Opie² described a case in which the pancreas was the seat of a remarkable lesion which appeared to involve chiefly the islands of Langerhans. Throughout the organ there were distinct, sharply outlined, hyaline areas embedded in the parenchyma. These areas varied in size and were most numerous in the tail. Histologically, they consisted of thick columns of a homogeneous, hyaline substance which was present outside the capillary endothelium

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The urine was examined once. It was amber in color, clear, and acid in reaction. Specific gravity 1025. Albumen and sugar were not present. Many pus and a few epithelial cells were found in the sediment.

A blood count showed moderate leucocytosis. Blood pressure, systolic 120, diastolic 80.

Necropsy Report. Necropsy begun one and a half hours post-mortem. On gross examination the viscera are not remarkable. The pancreas shows no macroscopic changes. The main pathology is in the cervical region where there is separation of the fifth and sixth vertebrae with rupture of the posterior longitudinal ligament and severe crushing injury of the cord with hemorrhage into its substance.

Anatomic Diagnoses: Myelitis, transverse cervical (traumatic); multiple hemorrhages into muscles and soft tissues of the back; multiple subpleural hemorrhages; chronic pulmonary tuberculosis; focal necroses of the liver; hyalinization of the islands of Langerhans; old, healed peritonitis, localized; congenital abnormality, of the pelves of kidneys; hydrocele.

Microscopic examination of the pancreas: The tissue is well preserved. Casually examined, the sections reveal little which appears to be abnormal. The acini stand out clearly and are well stained. In occasional small areas there is slight increase in the interacinar connective tissue, but in general the interstitial tissue is normal. The larger arteries are moderately sclerosed, but the smaller vessels are normal. The islands of Langerhans are clear and distinct and are of fairly uniform size, averaging 100 to 250 microns in the largest diameter. In the majority of these structures there are no visible histologic changes, but here and there one finds small groups of islets which show the characteristic hyaline deposit. Of 300 islands counted at random in five sections from different parts of the organ, 96 show the hyaline change, so that approximately one-third are involved. The appearance of these altered islets is as follows: A varying amount of homogenous, hyaline substance which stains pink with eosin is present within the affected structure. Occasionally the hyaline masses, which are usually round or globular, appear to lie between groups of islet cells, but most commonly they are in intimate contact with the endothelial lining of the capillaries. Isolated masses of the substance sometimes lie at the periphery of the island, in contact with the fibrous capsule of the structure. The de-

individuals consist chiefly of inflammatory lesions, in which leucocytic infiltration of the islands is present in the early stages, and moderate to advanced sclerosis in the late. Rarely hyalin also may be found in these late inflammatory lesions. Occasionally in young patients the islands show no noteworthy morphologic alterations, as was noted by Cecil in nine of twenty-six patients under forty-five. The usual lesion, however, is infiltration of the insular bodies with leucocytes, or replacement of the islet parenchyma with scar tissue. The hyaline change, then, is characteristic of a group of cases in which the duration of the disease has been of some length, usually more than three years, and in which the patients themselves are middle aged or older.

In only one instance have I been able to find any reference to hyaline changes occurring in the pancreas of a patient in whom diabetes mellitus was not present clinically. Ohlmacher¹¹ described a case, clinically non-diabetic, in which there was hyaline degeneration of the islets. Cirrhosis of the liver was also present. In this case there was great hypertrophy of the unaffected islets, a finding which Ohlmacher thought might account for the absence of glycosuria. With the exception of this single case, then, hyaline degeneration of the islands of Langerhans has always been definitely associated with diabetes mellitus.

In this paper I wish to report five cases which came routinely to necropsy at the Vanderbilt University Hospital, and which show varying degrees of hyaline degeneration of many of the pancreatic islands. In none of these patients were there symptoms of diabetes mellitus during life, and in only one case was a trace of glucose found in the urine on one examination. The individuals came to the hospital for various reasons, and the pancreatic findings were incidental. All of the patients were men over fifty years of age, and they thus fall into the age group in which hyaline degeneration of the islets in diabetics is most commonly found. The clinical histories and necropsy findings are outlined briefly below.

CASE 1. Clinical Report. V-25-5. G. R., a negro, married, farmer, 53 years of age, was brought to the hospital Nov. 1, 1925, following an automobile accident. A diagnosis of fracture of the sixth cervical vertebra was made. Below that level there was almost complete paralysis and loss of sensation. Laminectomy was performed the morning after admission, but 36 hours later his temperature rose, respiration failed, and he died quietly.

There was nothing remarkable in the patient's past history.

wall of the right ureter which is occluded by the growth. Hydro-nephrosis is present on this side. A large pelvic abscess, apparently due to postoperative infection, is also exposed. The other anatomic diagnoses are abscess of the cortex of the left kidney; chronic pyelonephritis, left; chronic cholecystitis; occlusion of cystic duct by calculus; chronic vascular myocarditis; generalized arteriosclerosis. No gross abnormalities of the pancreas are noted, save for slight firmness or toughness.

Microscopic examination of the pancreas: Sections from the pancreas show a moderate ingrowth of fat tissue between the lobules of the organ, and groups of fat cells are not uncommon within the lobules themselves. The interacinar connective tissue is greatly increased in amount in all of the sections. In some of the lobules the increase is slight, appearing merely as small strands of fibrous tissue which run diffusely through the lobule. For the most part, however, the sclerosis is so marked that in many lobules the acinar tissue is greatly atrophied or has disappeared, leaving small numbers of islets isolated and embedded in dense scar tissue. Small focal collections of lymphocytes are frequently seen. The fibrosis is definitely of the interacinar variety; and about the larger ducts there is little, if any, increase in stroma. Arteriosclerosis of the pancreatic vessels is moderately advanced; there being frequently a diffuse thickening of the intimal lining of the arterioles.

In the lobules where the chronic inflammatory process is not marked, the islets show no noteworthy changes. They are sometimes considerably larger than normal; one, for example, measures 602 by 376 microns. In general, however, they average from 250 to 350 microns in the greatest diameter, which is within the normal limits. In the areas where interacinar sclerosis is prominent, however, many of the islets show distinct changes. In many, about twelve to fifteen per cent of the total pancreatic islands, there is an increase in the connective tissue stroma; that is, the islets themselves are the seat of moderate to advanced sclerosis. In several sections one may find occasional islands which, except for a few atrophic parenchymal cells compressed between strands of collagen fibrils, are entirely replaced by scar tissue. These, however, are relatively uncommon. In addition to this insular fibrosis, about ten per cent of all the islets show the typical hyaline deposit, with or without associated sclerosis. This lesion is less common than the

gree of degeneration varies. In some of the islets there are only two or three small globules of the hyaline material, which appears to have little, if any, marked effect on the insular cells. In others, where the lesion is somewhat more advanced, the hyalin is present in considerable amount. It often appears to replace many of the cells which gradually atrophy or disappear. The cells which persist are compressed into small areas by the hyalin. They stain deeply and contain small, dark nuclei. Rarely one finds an islet in which the lesion is markedly advanced. Here the hyaline material is present in large amount in irregular rounded masses or globules which are conspicuous for their association with capillaries. Endothelial cells are still preserved and occasional red cells are seen within the narrowed lumina of the vessels. Between the hyaline masses, occasional compressed, elongated, or fusiform, darkly stained nuclei of parenchymal cells are found. In no case, however, does one find the entire islet replaced by the homogeneous material.

CASE 2. *Clinical Report.* V-25-6. L. B. M., a widower, 69 years of age, white, who worked as a clerk, was admitted to the surgical service of the Vanderbilt University Hospital, Sept. 27, 1925, complaining of hematuria, and pain in the lower abdomen. His past history was negative except for nocturia 2 to 12 times in the past 5 or 6 years. The present illness began 3 months before admission, with the appearance of blood in the urine. At first this symptom was unassociated with pain, but in the two or three weeks preceding entry he suffered a great deal. There had been a loss of 35 pounds in his weight during this recent illness.

Physically he was an obese individual with no other abnormal findings except those referable to the urinary system. A large tumor mass in the bladder was found on cystoscopic examination. On Oct. 13, 1925, the tumor was exposed, cauterized, and implanted with radium needles. The patient did well for about three weeks when deep X-ray therapy was instituted. To this treatment he reacted violently and died Nov. 6, 1925 with symptoms of alkalosis.

The urine on many examinations showed a trace of albumen but no sugar, except in a single specimen, taken just before he was removed to the operating room, in which a trace of glucose was found. In color the fluid was light amber or pale yellow, and clear. Specific gravity 1020 to 1021. On one examination there were many red cells. Later pus cells and organisms were found.

The leucocytic count was consistently high, ranging from 14,000 to 15,000 per c.mm. Blood non-protein nitrogen slightly elevated. Blood pressure, systolic 195, diastolic 75. Wassermann negative.

Necropsy Report: Necropsy performed forty-five minutes post-mortem. A large tumor mass is found in the fundus of the bladder. It is necrotic throughout and its surface is ulcerated. This neoplasm definitely invades the perivesical tissues, and extends into the

jority of these structures show few, if any, noteworthy changes. Slight postmortem degeneration of some of the cells is sometimes seen, but otherwise these islets are not remarkable. Scattered about among these, however, are isolated islands or small groups of islands which show the characteristic hyaline change. In a total count of 300 of these bodies in several sections from different parts of the pancreas, ninety-four were found to show the hyaline change in some degree. Like those of Case 1, these islets contain varying quantities of hyalin. In general, the affected structures contain only small amounts of the substance and nowhere is the process advanced. There are no islands which are completely replaced by hyalin. The close relationship of the material to the endothelial lining of the capillaries is again observed. Several islets are found in which mitotic division of insular parenchymal cells is active.

CASE 4. Clinical Record. V-26-1. C. J. M., aged 57, married, white, locomotive engineer, was admitted to the surgical service of the Vanderbilt University Hospital, Dec. 5, 1925. The complaints were weakness, loss of weight, and swelling of the left chest; all symptoms of about a year's duration, with gradual increase in severity in the month preceding admission.

On physical examination the chest was found to be firm and flat to percussion, and breath sounds could not be heard. A diagnosis of carcinoma of the lung was made, and on December 8th, the patient left the hospital unimproved. At home he failed rapidly and died Jan. 2, 1926.

The urine was amber with a slight cloudy precipitate. It was neutral to litmus. Sugar and albumen were absent. A few pus cells were found in the sediment.

The blood examination was not remarkable except for moderate leucocytosis, about 17,400 per c.mm., of which 86 per cent were polymorphonuclear leucocytes. Blood Wassermann negative.

Necropsy Report and Anatomic Diagnoses: Necropsy performed two hours postmortem. Carcinoma of left lung with metastases to the pleura, oesophagus, liver, pancreas, adrenal, regional lymph nodes, and left femur; infarction of right lung; infarction of liver and spleen; focal necroses of liver; parenchymatous degeneration of kidneys; hypertrophy of prostate; thrombosis of left external iliac vein; arteriosclerosis, generalized.

Microscopic examination of the pancreas: Sections from various parts of the pancreas show tissues which are well preserved. The interlobular connective tissue is not increased in amount but in places it is edematous and infiltrated with large mononuclear leucocytes and lymphocytes. The walls of many of the arterioles are thickened and a dense, homogeneous, hyaline material is present in

sclerosis alone. The hyaline masses are situated characteristically just outside the capillary endothelium, and only occasionally does one find an islet in which the change has progressed far enough to obliterate the structure almost entirely. In its microscopic appearances the hyaline material resembles that already described. In a few islets typical mitotic figures are found as evidence of regeneration of the parenchymal cells.

CASE 3. Clinical Report. V-25-13. J. A. C., 54 years of age, white, married, mill worker, was admitted to the Vanderbilt University Hospital, Dec. 14, 1925, in a dazed condition. He had been suffering with mental symptoms for about two years following an accident to his head. For six weeks prior to entry he had had spells of unconsciousness alternating with convulsions, apparently the result of a more recent accident in which he fell through a trap door and dropped several feet. Personality changes had been so noticeable of late that the patient was brought to the hospital.

As a result of thorough study the patient was thought to have either an abscess or a tumor of the brain. Since the Wassermann tests of both blood and spinal fluid were negative, and the colloidal mastic test not abnormal, syphilis was not considered. An operation was considered inadvisable and the patient grew gradually worse, dying Dec. 30, 1925.

The urine was not remarkable. It was amber, clear, and acid in reaction. No sugar was found. Albumen was not present. The sediment was negative.

The blood showed moderate anemia, 3,720,000 red blood cells per c.mm., and marked leucocytosis, from 14,000 to 24,000 per c.mm. 80 per cent of these were polymorphonuclear leucocytes. Blood pressure, systolic 135, and diastolic 80.

Necropsy Report: Necropsy performed about six hours post-mortem. On section of the brain a large, necrotic, edematous, cystic tumor (glioma) involving the basal ganglia and internal capsule on the left side is exposed. The remaining anatomic diagnoses are: Acute bronchopneumonia, terminal; central necrosis of the liver; parenchymatous degeneration of the kidneys; emphysema of the lungs; hypertrophy of the myocardium; adhesive pleuritis. The pancreas is not remarkable in gross.

Microscopic examination of the pancreas: Sections from the pancreas show moderate ingrowth of fat tissue between the lobules. There is no appreciable increase in the interacinar connective tissue, and evidence of an inflammatory process is lacking. Lobulation is distinct and the interlobular stroma is not increased. The tissue is well preserved except for occasional small foci of early postmortem degeneration. There is moderate arteriosclerosis of the smaller arteries. The islands stand out clearly in all sections. They are not enlarged nor do they appear to be increased in number. The ma-

Necropsy Report and Anatomic Diagnoses: Necropsy performed eighteen hours postmortem. Acute purulent meningitis, with considerable softening of the cerebral cortex; chronic vascular nephritis; passive congestion of the lungs; arteriosclerosis.

Microscopic examination of the pancreas: Sections from various parts of the pancreas show advanced postmortem degeneration which affects the entire organ. There is no increase in the interlobular connective tissue, but in places the interacinar stroma is considerably thickened and in these areas the acini are atrophied but the islets are not apparently affected. Foci of intimal thickening in the larger arterioles are seen. In general the insular bodies show little other than the changes associated with postmortem degeneration and a slight tendency to hypertrophy. Small numbers of the structures are definitely enlarged, two measuring, for example, 520 by 356 microns, and 465 by 210 microns, respectively. By far the larger number of islands, however, fall within the normal size limits. Here and there the typical hyaline deposit is noted. Out of 300 islets counted in several sections from various parts of the organ, hyaline material is found in only ten. About three to four per cent of the total islets are, therefore, involved. In no case is the hyalin present in large amount, not more than two to five small masses or globules being found in any one island. In its essential characteristics the hyaline material resembles that already described.

DISCUSSION

Five cases are presented in which hyaline degeneration of the islands of Langerhans was found on histologic examination, but in which, during the lifetime of the individuals, there was no clinical evidence of diabetes mellitus. The patients came to the hospital for various reasons, three because of malignant disease, one as the result of an accident, and one with acute meningitis. Urine examinations in all cases but one were negative for glucose. This one patient (Case 5), showed a trace of sugar in the admission specimen, which was the only one obtained. In Case 2, a single test made just before the patient went to the operating room, revealed a trace of sugar, but many other examinations were consistently negative. Since, under conditions of undue nervous stress, glycosuria may be a physiologic phenomenon, little attention was paid to this isolated finding.

the intima. In two sections, cords of atypical epithelial cells are found in some of the lymphatics, evidently a metastatic growth from the tumor of the left lung. The tumor, however, does not damage or destroy any of the pancreatic parenchyma. The pancreatic ducts are not remarkable except for occasional small, irregular concretions which are present in some of the lumina.

The interacinar connective tissue is not remarkable. It is not increased in amount and only in rare, inconspicuous foci does one find a slight chronic inflammatory reaction as indicated by the presence of small numbers of lymphocytes. The acini in general show no histologic abnormalities.

The islands of Langerhans are clear and distinct. The majority of these structures show no histologic changes but, as in Cases 1 and 3, varying degrees of the hyaline degeneration may be found in small numbers of them. In a count of 300 islets, sixty-two are found to contain some degree of the hyaline deposit, so that approximately twenty per cent of the structures are involved. The appearance of the hyalin varies in no way from that found in the other pancreases already described. In general, the affected islets contain only a small amount of the deposit, and no completely hyalinized islets are found. In size the islands are not remarkable. The largest structure in which hyalin is found measures 400 by 250 microns. The islets which are not involved vary somewhat in size, but only rarely does one encounter a structure which is beyond normal limits. The largest structure found measures 411 by 361 microns, and this is only slightly enlarged. A careful search for evidence of regeneration of the island cells fails to disclose mitotic figures.

CASE 5. *Clinical Report.* V-26-20. J. R., a negro, laborer, aged 55, was brought to the hospital, April 3, 1926, in a state of delirium. For several days previously he had felt sick, and on the day of admission he became suddenly unconscious while at work.

The physical examination showed a fairly well developed but rather poorly nourished adult negro in deep coma. Deviation of the eyes to the right, no reaction of pupils to light, a smooth right face, spasticity of upper extremities, tortuosity and thickening of peripheral vessels, and a blood pressure of 220 systolic, and 110 diastolic, led to a diagnosis of cerebral hemorrhage.

The patient was given morphine and a tap-water drip was started, but no improvement resulted. The coma grew progressively deeper and he failed rapidly, dying five hours after admission.

A single urine examination showed a considerable amount of albumen and a trace of sugar. A few granular casts and pus cells were found in the sediment. The fluid was straw-colored with a specific gravity of 1026.

Islands of Langerhans. In all five cases there is definite evidence of hyalinization of many of the islets. As studied in hematoxylin-eosin preparations, the hyaline material stains clearly pink and appears usually as a homogeneous, structureless substance. At times, however, the material seems to be arranged as fine, radiating fibrils which run from a central core and which give to the mass the appearance of a minute fuzzy ball. Occasionally, also, the material assumes a foamy appearance, due to the presence within it of numerous small vacuoles. These morphologic variations appear to be artifacts, since the greater part of the substance is amorphous, structureless, and smooth. Nearly always the hyaline masses are contained within the insular structures, only rarely being found outside.

The appearance of the islets which contain this material varies. Those which show the simplest type of involvement contain only one or two small masses of hyalin. These masses are present immediately outside of and adjacent to the endothelial lining of the local capillaries. The endothelial cells themselves are usually clear and distinct, no malformation being noted. The hyaline substance appears to rest within a small but definite space which is bordered on one side by endothelium, and on the other by islet cells. The islet cells are not abnormal except for slight pressure atrophy. The hyalin in most cases is entirely separate and distinct from the parenchymal cells, the borders of which are visible but often appear frayed and irregular.

Where the insular involvement is marked, the hyaline masses are more numerous, but they differ in no whit from those already described. About the capillaries the substance accumulates, forming a sort of cylinder which sheathes the vessel. In cross-section, then, the material is arranged in a circular fashion, in some places resembling a smooth, homogeneous ring; in others, where the substance is gathered into minute globules or spherical masses, appearing as a series of droplets encircling the vessel (Fig. 3). Where the capillaries are cut tangentially, the hyaline material assumes various shapes and forms, according to the plane of section. When the vessel is cut longitudinally, for example, the material is stretched out as irregular, wavy, homogeneous columns which run parallel with the blood channel (Fig. 1). It appears therefore that the hyalin collects chiefly about the capillaries of the tuft and, gradually accumulating, presses upon and causes atrophy of the parenchymal cells.

It is a noteworthy fact that all of the patients were over fifty years of age. This agrees with the findings of Cecil, Warren and Root, Opie,¹² and others, who, as has already been indicated, found in diabetics by far the greater percentage of hyaline degenerative changes of the pancreatic islands in adults over forty-five years of age.

These cases are taken from a series of eighty-four routine necropsies made at the Vanderbilt University Hospital during the period from Oct. 1, 1925, to Oct. 1, 1926. The pancreatic changes were found during microscopic study of the organs. In each case the pancreas revealed little on careful gross examination. In one of the cases (Case 2), which showed marked interacinar sclerosis, the organ was described as slightly firm and tough. The others were essentially negative, except for advanced postmortem changes in one (Case 5). The tissues were fixed in Zenker's fluid, several sections being taken routinely from the head, body, and tail of each pancreas, so that fair samples of the organs were available for microscopic examination. Five or six blocks of tissue, one or two from each part of the organ, were selected for embedding, and many sections were prepared.

MICROSCOPIC EXAMINATION. *Acinar tissue and stroma.* In three of the five cases no changes are observed in the acinar tissue. The glands are histologically normal. In the two remaining cases, however, where interacinar sclerosis is marked, there is atrophy and disappearance of much of the glandular tissue, accompanied by thickening and condensation of the surrounding stroma. In these cases there is further evidence of a chronic inflammatory process as shown by a mild infiltration of the connective tissue septa with cells of the lymphocytic series. In one case (Case 5), postmortem degeneration is so marked that a study of the acinar tissue cannot be made.

Blood Vessels. Arteriosclerosis is a common finding in all the patients, and intimal changes in the pancreatic arteries are moderately marked. In two cases there is considerable hyaline thickening of the intima of the smaller arteries, but this homogeneous material does not react to the various stains as does the hyaline substance which is found in the islets. The production of the insular hyalin is not dependent, as far as can be determined, on the arteriosclerotic process. The vascular changes appear to be merely coincidental and not in any sense causal.

which this degenerative process is taking place are stained a light blue color with Mallory's aniline blue, and resemble in a way the denser accumulations. Ultimately there is complete transformation of the parenchymatous cells, and the islet becomes a hyaline mass in which there remain only the altered capillaries.

In this study, many islands were carefully examined for the appearance of epithelial cell degeneration resembling that described by Opie. Here and there in some of the islets there appear to be occasional isolated cells which contain minute homogeneous masses which stain faintly blue by the aniline blue method of Mallory. These cell changes resemble very much the appearance described by Opie in his report of studies on sections stained by the same method. In general, however, these isolated, blue-stained, hyaline foci are clearly outside of epithelial cells, and appear to rest in small, definite intercellular spaces. Cell borders are often indented to form these openings, and the appearance is quite similar to that found in the liver in cases of chronic biliary stasis, where small globules of bile fill the distended intercellular ducts. These minute hyaline masses, when followed in serial sections, are commonly found to be portions of larger accumulations which are present either above or below the plane in which the isolated masses appear. There is thus but little evidence of a protoplasmic transformation within the insular cells, and the small isolated globules of hyaline material found in any single section are generally only portions of larger masses in another part of the islet.

The majority of the hyaline masses, however, are present about the capillaries, and they appear morphologically to be the result of a slow change which occurs in the fibrous tissue surrounding these vessels. The steps in the process are hard to follow. The first evidences appear to be the deposit or formation of a thin layer of homogeneous, hyaline material in the fibrous tissue adjacent to the endothelial lining of the capillary. This slowly accumulates and thickens up, gradually pushing the epithelial cells away. These cells undergo slow atrophic and degenerative changes, until, where the deposit is great, they ultimately disappear and are replaced by hyalin. This material, therefore, is formed outside the epithelial cells of the islets rather than from their cytoplasm. In spite of the reaction with picric acid and acid fuchsin, the substance seems to be a degenerative product formed as amyloid is, within the perivascular stroma.

Only rarely is the hyalin present in such amounts that it replaces half or more of the islet (Fig. 1); and no completely hyalinized islands are found. Where the involvement is most marked, many of the parenchymal cells have atrophied, and some have disappeared. The cytoplasm of the atrophic cells stains deeply with eosin, and appears finely granular. The nuclei are small, pycnotic, and deeply stained with the basic dye. Chromatin granules are not usually visible, the nuclear stain being diffuse. The endothelial cells are flattened and compressed where the hyalin is present in moderately large quantity, and the capillary lumina are reduced in size. Rarely a completely collapsed capillary is found. In general, however, the hyaline degenerative process is not far enough advanced to produce marked capillary or parenchymal changes.

It seemed of interest to study more carefully the nature of this hyaline material, and sections from the various pancreases were subjected to a variety of staining methods. The material was uniformly negative when stained for amyloid with iodine-green or methyl violet, and amyloid was, therefore, readily ruled out. The reaction of the material to hematoxylin-eosin has already been mentioned. With eosin-methylene blue the substance stains deeply and homogeneously pink. By the use of Mallory's phosphotungstic acid hematoxylin the hyalin takes a light orange-brown stain, and stands out clearly from the surrounding purplish blue parenchymal cells. Stained by the aniline blue method as employed by Mallory for the demonstration of collagen, the hyalin takes a deep blue color, and forms a brilliant contrast from the bright red counterstain of the surrounding cells. The material, when stained with Van Gieson's picric acid and acid fuchsin mixture, takes the yellow color, rejecting the fuchsin. It thus behaves, according to Ernst, as does hyalin of epithelial origin. This homogeneous substance, therefore, stains exactly as did the hyalin described by Opie in the pancreases of diabetic individuals, and it may be reasonably assumed that we are dealing here with the same substance.

The hyaline material, according to Opie,¹² is of epithelial origin, the degenerative process first manifesting itself by changes in the protoplasm of the islet cells. Subsequently the cytoplasm becomes homogeneous, the nucleus disappears, and small particles of this altered, structureless cytoplasm fuse to form larger masses of hyalin which lie in contact with the fibrous septa of the islet. The cells in

more common to find a larger proportion of affected than unaffected structures. Here, however, approximately two-thirds or more of the insular bodies are not morphologically abnormal.

Warren and Root, in studying the pancreases of diabetics who had been treated for a certain period of time with insulin, indicate that, in their opinion, regeneration of island cells can occur; and they cite cases of hemochromatosis and acute lobar pneumonia in which active regeneration is evidenced by the presence of one or more mitoses in single islets. In true uncomplicated cases of diabetes they found no evidence of regeneration. This they feel is due to the ex-

TABLE 1

Summarizing the Findings of the Five cases Reported in this Paper

Case	Name	Age in years	Per cent of islands involved	Hypertrophy of uninvolved islands	Evidence of regeneration	Fibrosis of islands	Interacinar sclerosis	Arterio-sclerosis
1	G.R.	53	32	None	None	None	Very slight	Slight
2	L.B.M.	69	8-10	Slight	Slight	12-15%	Marked	Moderate
3	J. A. C.	54	31	None	Slight	None	Very slight	Moderate
4	C.J.M.	57	20	Very slight	None	None	Very slight	Moderate
5	J.R.	55	3-4	Moderate	None	None	Moderate	Slight

treme chronicity of the course of the disease, where attempts at regeneration would be slow, and where mitotic figures would hardly be expected. Boyd and Robinson¹³ also reported one case of diabetes in a boy who was accidentally killed after six months of insulin treatment, in whose pancreas there was definite evidence of regeneration.

It was of interest, therefore, to study the sections at hand from these five patients with a view to discovering whether or not insular regeneration was evident. In the majority of cases no mitotic figures within the islands could be found on careful high power study. In sections from the pancreases of Cases 2 and 3, however, several mitoses were discovered. These were evident not only in islets which were free from the hyaline deposit, but also in those in which

The islets which are here involved show, therefore, slight to moderate hyaline degeneration. No structures are completely destroyed, and the pathologic process, morphologically, appears to be early. From islets in which two or three small droplets of hyalin are present, there are all degrees of involvement to those in which approximately one-half to three-quarters of the structure is replaced by the homogeneous material. These latter present the most advanced form of the lesion. In spite of atrophy and even disappearance of many of the islet cells, every injured islet studied contains some parenchymal cells which are not markedly abnormal.

In studying the pancreases of ninety cases of diabetes mellitus, Cecil found that twenty-seven showed the hyaline change. Twenty-one of these patients were 48 years of age or over, and thus fall into the age-group which most concerns us here. Of this latter group the degenerative lesion in the pancreases of seventeen was associated with varying degrees of chronic interacinar pancreatitis, while four showed no changes in the acinar parenchyma or stroma. In my five cases there are two which show marked interstitial sclerosis, but in only one of these does the scarring affect the islets themselves. In this case (2), insular fibrosis is more prominent than the hyaline accumulations, appearing in twelve to fifteen per cent of the structures, while hyalin is present in about eight to ten per cent. Rarely both lesions are observed in the same islet. In three cases, the hyaline change is relatively uncomplicated, only minute foci of interacinar fibrosis being occasionally found.

The relative number of islets involved in each of these cases is noteworthy, especially when considered from the standpoint of the relation of the hyaline degenerative process to diabetes mellitus. To get an approximate estimate of this involvement the following procedure was carried out. Three hundred islets, taken at random from the various sections available in each case, were counted and carefully studied. All structures showing even the slightest degree of hyaline deposit were noted and recorded. In Table I the relation of involved to uninvolved islets is indicated. One finds, as a result of this study, that in no case is more than one-third of the total number of islands affected by the degenerative process. In three cases considerably less than a third give evidence of the deposit. This fact again seems to point to an early stage in the process, since, in the majority of diabetic pancreases which show this type of change, it is

Cecil, in his study of ninety cases of diabetes mellitus, considers 400 microns as the upper normal limit, and that dimension is used as a criterion here. Ohlmacher considered all structures measuring more than 300 microns in size as definitely enlarged.

Using a Zeiss ocular micrometer which had been previously standardized against a stage micrometer graduated in hundredths of a millimeter, the islands in all of the pancreases studied were accurately measured. In two of the cases (1 and 3), which show the largest percentage of hyalinized islets, no hypertrophied bodies are found. In the other three cases, however, small numbers of enlarged islands are present, the largest found measuring 602 by 376 microns (Case 2). In Case 4 the largest structure is but slightly beyond the normal limits, measuring 411 by 361 microns. In the last case a number of hypertrophied structures are present. In general the enlarged islets are free from the hyaline deposit. A careful search for mitoses within these bodies was unfruitful. One may conclude from these findings that hypertrophy of uninvolved islets does occur to a certain degree. Since, in these cases, a far smaller number of islands are affected by the hyaline degenerative process than were found by Ohlmacher in his case, the need for increased insular tissue is less marked.

It is evident that in the pancreases here described there is a slow, progressive, degenerative change which affects specifically the islands of Langerhans. Varying numbers of these structures contain a hyaline substance similar to that which other workers have described in the pancreases of diabetics. These other investigators have shown that the lesion is one which develops usually in later life and that only a limited number of cases of diabetes mellitus are ascribable to it. It is brought about gradually, and generally by conditions which are incident to the fifth and sixth decades, and its cause is entirely obscure. In my cases a relatively small number of the insular bodies are involved, and then only to a moderate degree. The change appears to be progressive, and if continued long enough would result in sufficient injury to the islets to cause a physiologic deficiency in the normal hormone, thus bringing about the clinical disease. It is believed that such is the course of events in cases of diabetes in which the islands, histologically, show the hyaline change. In the cases here reported the definite degenerative change is progressing in the islands, and in two cases definite evidence of active regeneration

the degenerative change was relatively far advanced. The mitotic figures were definitely within the parenchymal cells of the islands, and could easily be distinguished from regenerative changes of the endothelium. The presence of this activity in the insular tissue shows the capacity of the islet cells to regenerate when injury has taken place, but does not, in either of these instances, prove that the regeneration is the direct result of a physiological deficiency in islet tissue alone. The necropsy findings in both of these cases showed evidence of acute infections. In Case 2 there was a large malignant tumor of the bladder with a secondary infection of the operative wound. This infection was acute, and blood stream invasion was indicated by infectious lesions in the kidney. Case 3 showed acute bronchopneumonia, with a moderate degree of toxic central necrosis of the liver. It may reasonably be assumed, therefore, that the injury in each case which resulted in regeneration was the acute toxemia rather than the slow, progressive insular change which was also present. Whether or not the considerable reduction in the amount of normal insular tissue available in these pancreases, has any effect on the readiness with which active regeneration will take place following a secondary acute toxic injury, cannot be definitely stated. It is not unreasonable to assume that, with a large number of islets seriously impaired by a hyaline or sclerotic change, the remaining normal tissue, if injured acutely, might regenerate more actively than the insular tissue in a pancreas which was otherwise normal. Of greatest importance here, however, is the fact that regeneration is evident in pancreases in which the characteristic hyaline degenerative process is relatively advanced, even to the point where new cell formation is observed in islets which themselves show the progressive, destructive change.

Ohlmacher, in reporting his case of hyalinization of the pancreatic islets without accompanying diabetes, calls attention to the large number of greatly hypertrophied islands. A careful study of the size of these structures was made in the present instance, and although occasional enlarged islands were found, the number which have undergone hypertrophy is not great. Further, some differences of opinion exist with regard to the size of normal islets. Koelliker,¹⁴ reports the average size of the structures as from 70 to 300 microns. Laguesse,¹⁵ on the other hand, states that normally many of the islets may measure as much as 400 microns in the greatest dimension.

3. The character of the lesions suggests a toxic origin, the injurious agent acting slowly and progressively over a very long period of time.

4. The degenerative process in each case affects less than one-third of the islets studied.

5. Tintorially and chemically the hyaline substance resembles that described by Opie and others in cases of diabetes mellitus studied by them.

6. The material appears to be laid down outside the blood vessel endothelium, in the subjacent connective tissue, and is not a product of epithelial cell degeneration.

7. In two cases there is evidence of regeneration of the islet cells, not only of unaffected but of affected islets.

8. Moderate hypertrophy of some of the uninvolved islets is noted in three cases, but this bears no constant relation to the amount of insular tissue involved in the degenerative process.

9. One case shows moderate sclerosis of the islets in addition to the hyaline change.

10. Only two cases show a noteworthy increase in the interacinar connective tissue.

CONCLUSIONS

1. Hyaline degeneration of the islands of Langerhans is a lesion which develops commonly in later life and only a small number of cases of diabetes mellitus are ascribable to it.

2. It appears to be the result of slow toxic injury in which the hyaline material gradually accumulates, injures and finally destroys the islet cells, ultimately replacing the insular tissue. Regeneration takes place, but the regenerated tissue, under the influence of the continued action of the injurious agent, is again slowly destroyed. Gradually the regenerative power of the pancreas is worn down, and finally, when so much islet tissue is destroyed that the normal glycolytic hormone can no longer be supplied in sufficient amount, the phenomena of diabetes mellitus appear.

3. These cases may be classed as falling into a prediabetic group, sufficient insular tissue not being involved to bring about the clinical symptoms of diabetes mellitus.

of islet cells is demonstrated. In three of the cases hypertrophy of moderate numbers of uninvolved structures is also evident. These facts, together with the finding that less than one-third of the insular bodies are involved, and these to a moderate degree only, permit one to infer, I believe, that the degenerative process, whatever its nature may be, which causes the hyaline material to form in the insular structures, precedes the breakdown of the normal carbohydrate metabolic mechanism. These observations thus form another link in the chain of evidence which points to the part played by the islands of Langerhans in the utilization of sugar. Unquestionably in cases of late diabetes, where hyaline islets are found in great number, there must have existed a prediabetic period, in which slow changes in the islands, similar to those here described, took place. During this time some injurious agent, probably toxic in character, as suggested by Warren and Root, causes the slow formation or deposit of hyalin in the island. As the hyalin increases in amount it advances upon the normal parenchyma, causing atrophy and ultimate disappearance of the island cells. Since the process is very slow, the cells may regenerate, as has been demonstrated here. With the continued progress of the injury, however, the regenerated cells, as well as those which previously existed, are finally destroyed. With the advance of this process, there is slow and gradual destruction of more and more insular tissue until a point is reached where a sufficient amount of active islet parenchyma to produce the glycolytic hormone is not available. There then appear the phenomena of hyperglycemia and glycosuria. Concerning the regeneration of totally new islets, as suggested by Warren and Root, nothing can be said from the present study. That active regeneration may take place within the islands themselves is unquestionable, but whether or not new islands arise cannot be stated.

SUMMARY

1. There are here reported five cases, clinically non-diabetic, in which the islands of Langerhans show the hyaline degenerative change characteristic of certain cases of diabetes mellitus.
2. All of these patients were over fifty years of age and thus fall into the age-group in which hyaline degeneration of the islets is commonly found in true diabetics.

DESCRIPTION OF PLATE

PLATE 125

FIG. 1. Case 3. Mallory's aniline blue stain. Small islet showing hyaline masses grouped about capillaries. One vessel, cut longitudinally, shows the wavy, hyaline columns running parallel with the wall. $\times 500$.

FIG. 2. Case 1. Mallory's aniline blue stain. Islet showing several hyaline masses. $\times 500$.

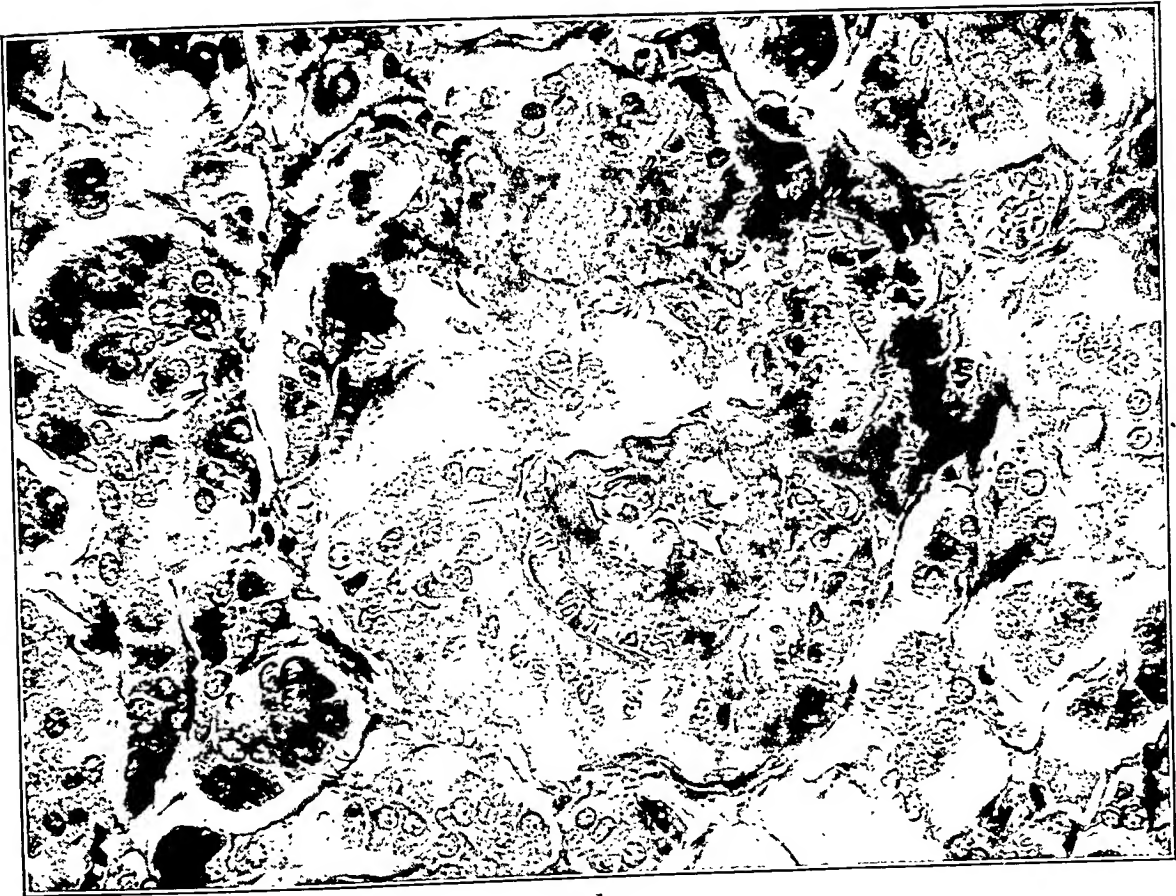
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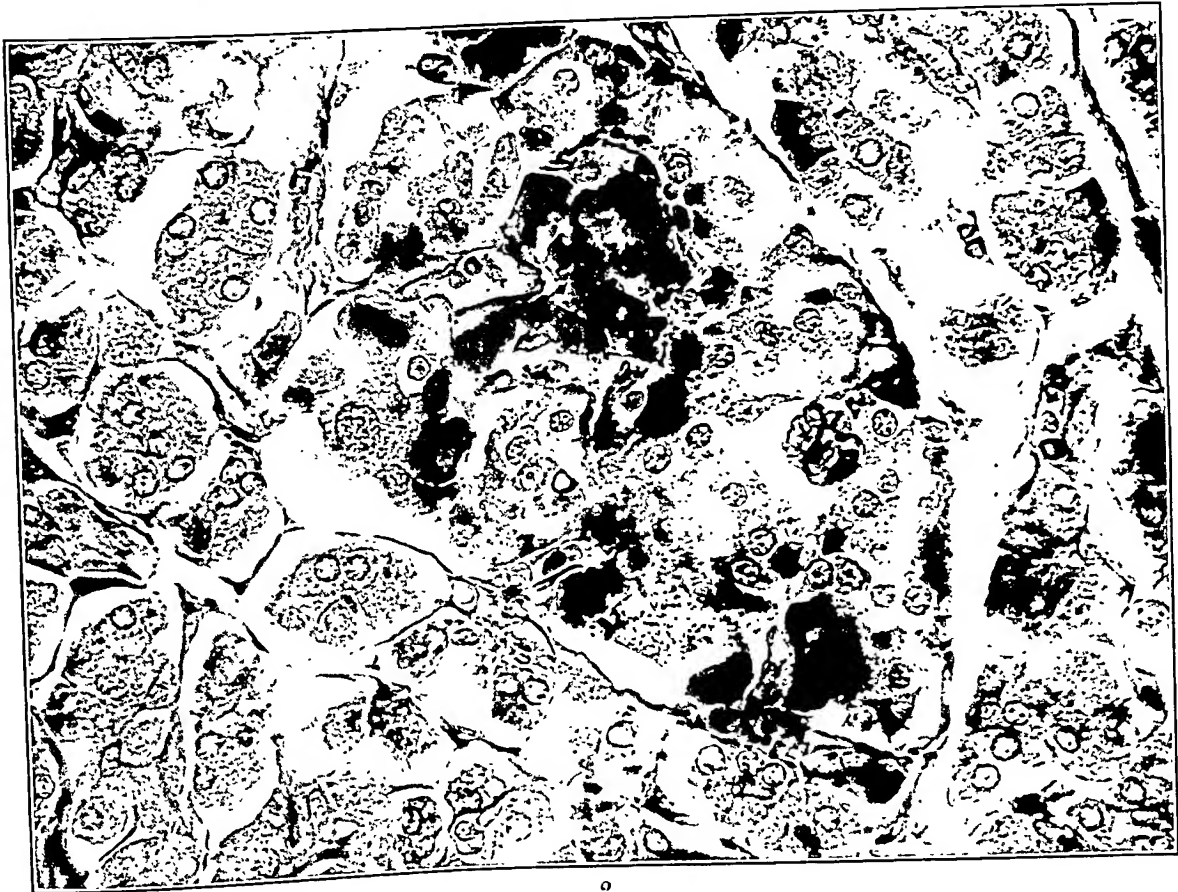
PLATE 126

FIG. 3. Case 3. Mallory's aniline blue stain. Island of Langerhans showing a thick collar of hyalin encircling a small capillary. $\times 500$.

FIG. 4. Case 4. Mallory's aniline blue stain. Small island with multiple hyaline masses. $\times 500$.



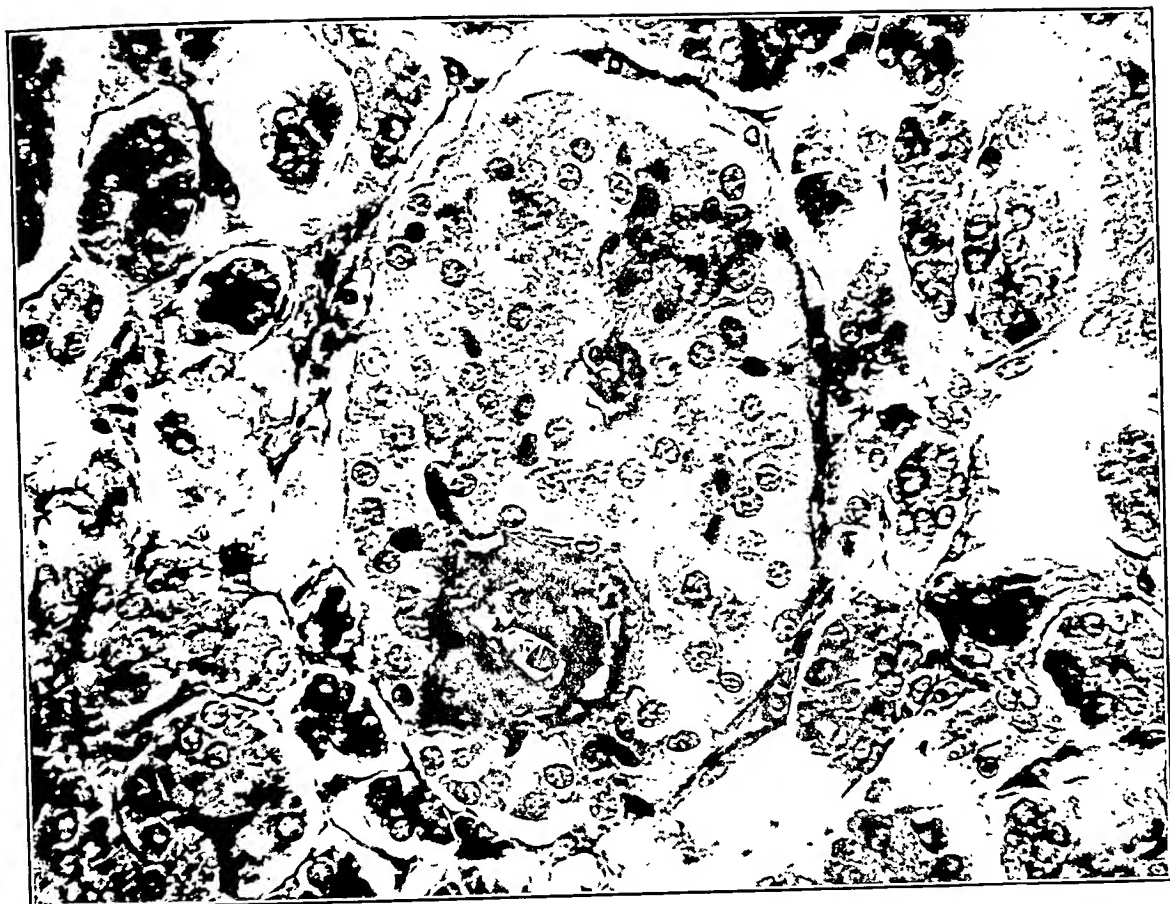
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PLATE 127

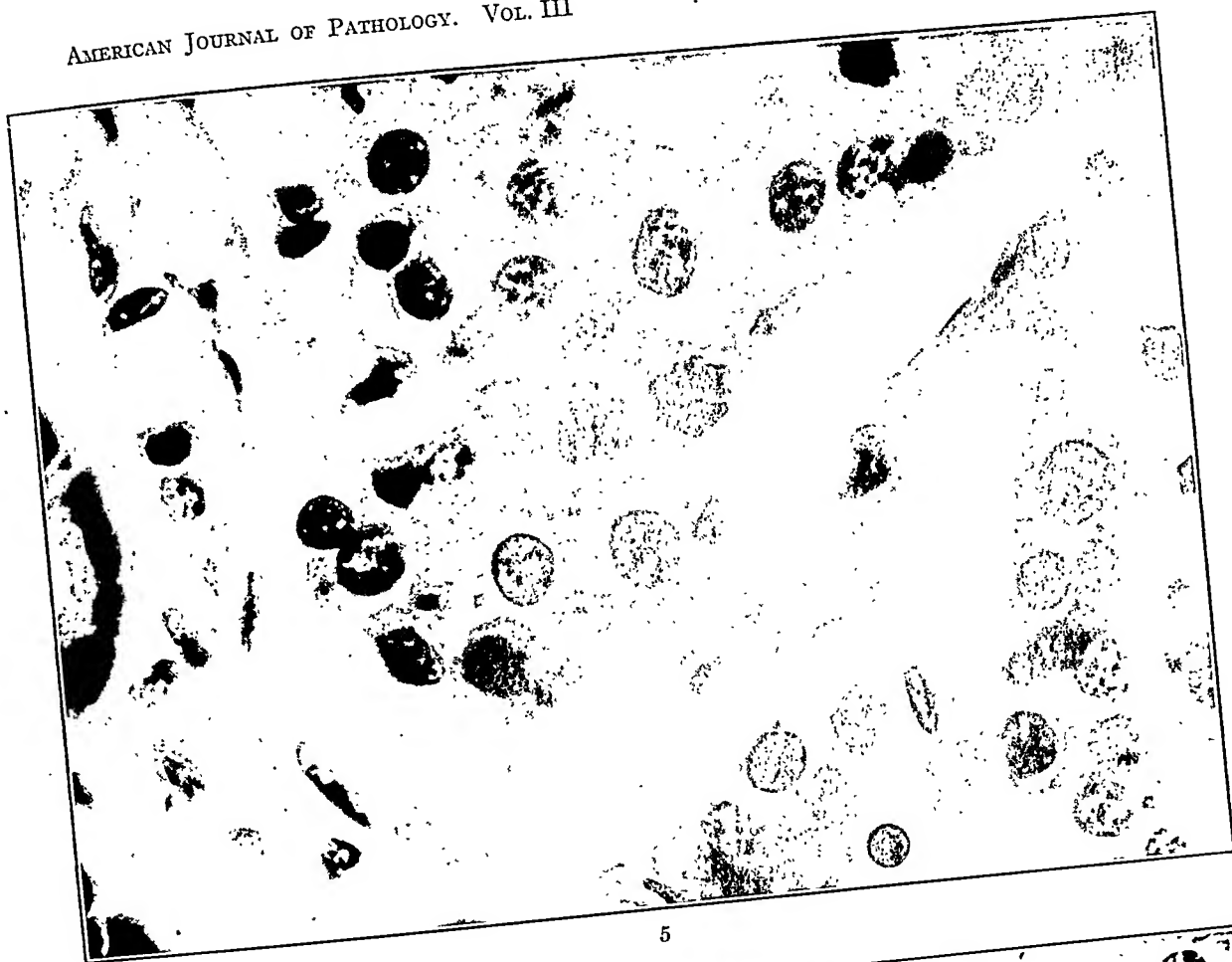
- FIG. 5. Case 2. Hematoxylin-eosin stain. One mitotic figure in an islet which contains none of the hyaline deposit. $\times 1000$.
- FIG. 6. Case 3. Hematoxylin-eosin stain. Mitotic figure in parenchymal cell of an island containing multiple hyaline masses. $\times 1000$.



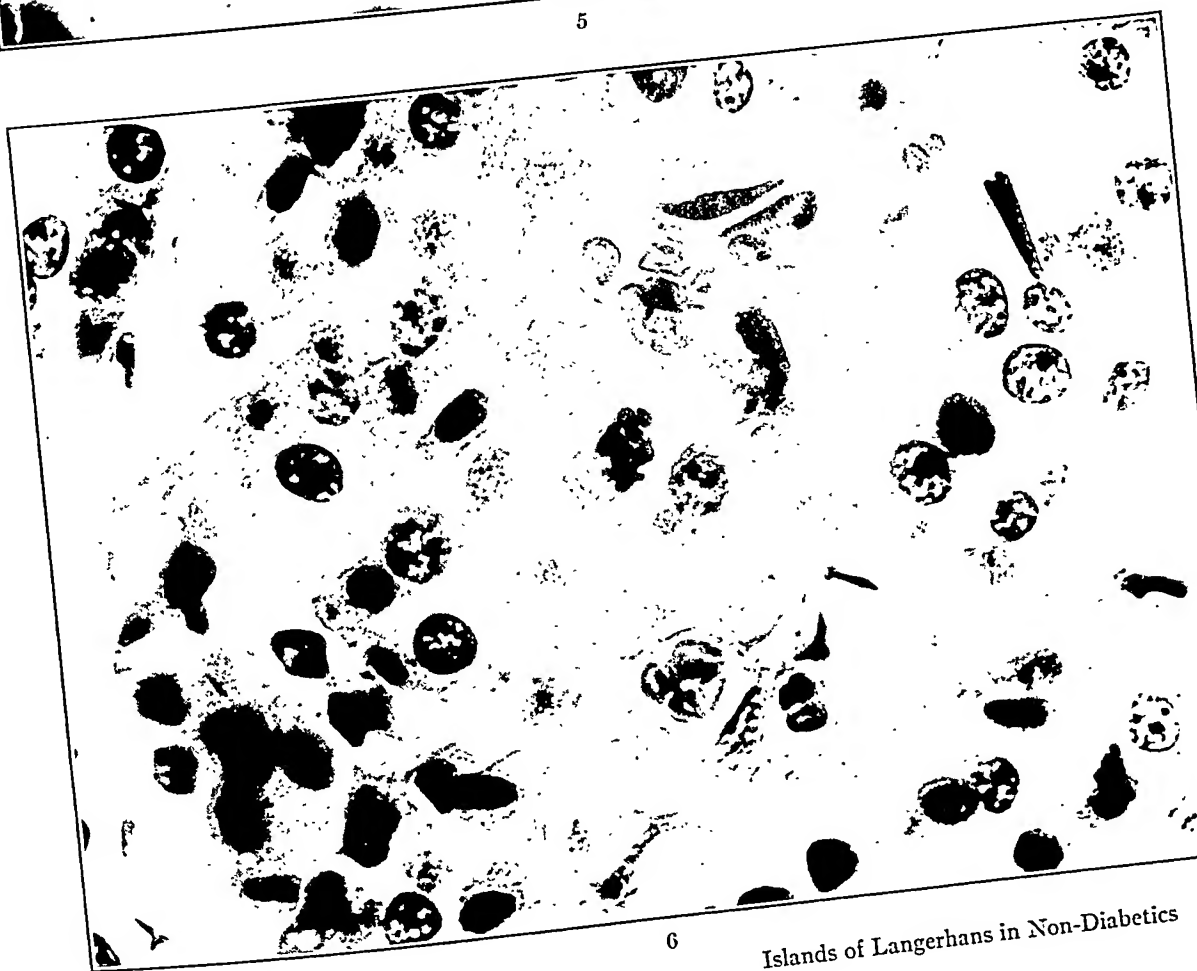
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Islands of Langerhans in Non-Diabetics

It is the usual finding in yellow fever that the spleen is neither altered in its size, nor are definite macroscopic changes sufficiently pronounced to attract attention. Nevertheless on closer examination the splenic structures are found to have suffered changes which are sufficiently distinctive to be of diagnostic value in distinguishing the lesions from those arising in other tropical diseases with which yellow fever may at times be confused. For this reason, I draw attention to certain alterations which I have observed in the splenic structure in cases of yellow fever occurring in West Africa. It is, however, also important to point out that these changes which appear in the spleen are also encountered in other acute intoxications both bacterial and chemical, in which, however, liver necroses are wanting.

These studies are based on a series of thirty-five cases of yellow fever which came to necropsy in Nigeria and the Gold Coast in 1926 and 1927. We are indebted to Dr. Henry R. Muller of the Yellow Fever Commission, Dr. Andrew Connal, Director of Medical Research, Nigeria, and to Dr. W. A. Young, Director of the Pathological Laboratories, Gold Coast, for placing their materials at our disposal. Of the thirty-five cases, twenty-eight occurred in European whites, and seven in native blacks. Death took place from the third to the tenth day after the beginning of the illness. In all of them the clinical diagnosis of yellow fever was substantiated in the pathologic analysis of the organs.

The macroscopic examination of the spleen offers little evidence of changes occurring within its structure. The spleen is commonly of normal size, usually quite flabby and dark in color. On section it is noted that the pulp is markedly congested and the tissues are quite friable. The malpighian bodies are visible as small greyish areas and may appear more prominent than normal. At other times again, the malpighian bodies are blurred, and rather diffuse in their periphery, having lost their punctate appearance. Necroses are never visible to the naked eye. The appearance of the spleen may be altered by the presence of changes due to chronic malaria, which is usually prevalent in yellow fever areas. However, in those individuals who have recently arrived in the infected zone, and in whom such added lesions are not present, the characteristic lesions are fairly constant.

The microscopic examination shows the sinusoids of the pulp sub-

THE SPLEEN IN WEST AFRICAN YELLOW FEVER *

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Although the literature on yellow fever is fairly extensive and we have a number of reports on the pathologic changes which arise in the internal organs, very little comment has been made on the changes which may be noted in the tissues of the spleen. In the studies which have been carried out on postmortem material the greatest attention has been given to the heart and liver. This might be expected inasmuch as the clinical manifestations arising in yellow fever are, to a great extent, concerned with lesions in these organs, and the most prominent pathologic changes are those which appear in the hepatic tissues. The best discussions on the microscopic pathology of yellow fever have been given us by Councilman,¹ Rocha-Lima,² Seidelin,³ and Marchoux and Simond.⁴ It is evident from all of these studies that the injury done to the tissues in yellow fever is dependent on a severe intoxication in which a fatty degeneration and necrosis makes its appearance in the organs. The liver and kidney suffer most severely, and many other tissues are similarly involved but play a minor rôle in bringing about the clinical manifestations. In none of these tissues is there evidence of a primary inflammatory response, nor is it evident from the histologic characters that the degenerative processes are directly referable to the presence of the infectious virus in the involved areas. It would seem that the intoxication which involves the various structures in the body is brought to them by the blood stream, being produced at an undetermined site. Since this intense intoxication often leads, within a few days, to an almost complete necrosis of the liver, it would seem that the poison, as well as the virus, is present in the circulating blood and has its influence on all susceptible organs. This toxin, which has not been isolated, does not produce similar lesions in the experimental animals which have been tested.

* The observations on which this paper is based were conducted under the auspices of the International Health Board.

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These endothelial cells vary somewhat in size; most of them are spherical but some are elongated or distorted. Turnbull,⁵ who has also commented upon the presence of these cells, refers to them as free endothelial cells. They possess phagocytic qualities and we have noted that in some cases they suffer a granular and, at times, hyaline degeneration similar to those which lie within the midportion of the lymphoid follicles. A similar finding has been reported by Durham⁶ who, in describing the spleen, states that "the marked feature is the large number of active macrophage cells in the pulp; some much vacuolated and most containing remnants of nuclear material."

In the later stages of the reaction, the endothelial cells occurring in the germinal centers of the malpighian bodies show hyaline changes and a fusion of their structures so that little remains of the original germinal centres. Towards the periphery of the follicle the degeneration of the endothelial cells is not of this type, or as marked, but the individual cells show a primary enlargement and granular degeneration of the cytoplasm, with subsequent death of the cells and a formation of protoplasmic debris. This endothelial degeneration in the outer zones of the follicle frequently gives rise to very bizarre types of cells varying from large multinucleate forms to irregular fragmentations which are partly phagocytosed by the neighboring reticulo-endothelium. Associated with these evidences of degeneration in the endothelial cells there are also some cells exhibiting mitoses of their nuclei. The mitotic figures appear as monasters or diasters, but the chromatin material which is arranged in rods appears very dense and without the delicate structure of normal mitoses. Furthermore, these cells exhibit areas of degeneration in their cytoplasm which assume a hyaline and somewhat acidophilic character. It appears to us that these mitotic processes do not indicate a normal process of cell division, but one of nuclear response of a degenerating cell.

The lesions in which these peculiar endothelial changes are most prominent are in the borders of the follicles where the funnel-shaped extremities of the efferent capillaries are arranged about the follicles. These have been described by Robinson.⁷

During the process of degeneration of the endothelial cells and subsequent to the enlargement of their structure, fragmentation of the nuclei gives rise to peculiar chromatin masses which may readily

stance widely dilated and engorged by red blood cells. There is rarely evidence of infiltration by polymorphonuclear leucocytes in the pulp tissues, and we observe an increase in these cells of the spleen only in cases where there is considerable necrosis of endothelial cells following hyperplasia. With the dilatation of the sinusoids, the endothelial cells appear to be crowded along the borders of these channels, and only occasionally are mild reactions of endothelial hyperplasia observed in the pulp areas. These endothelial cells show some phagocytic activity by the presence in their cytoplasm of occasional red blood cells and pink-staining debris from necrotic cells. The numbers of phagocytic cells or of loosely scattered large endothelial cells along the course of the sinusoids are never great.

On the other hand, more definite and constant changes are observed in the lymphoid follicles. These structures appear to pass through a sequence of changes, in the early stages of which there is some increase in the endothelial cells of the germinal centres. The lymphoid follicles of the spleen normally possess a small collection of endothelial cells in their centres, as well as a sprinkling of similar cells through the main lymphoid mass towards the periphery. The early reaction in the moderately intense cases of yellow fever is one in which the follicle appears somewhat enlarged through the hyperplasia of the endothelial elements. This is soon followed by a diminution in the size of the follicle resulting from the loss of the lymphocytes which disappear from the spleen structure entirely. These lymphocytes not only diffuse through the spleen pulp, but also disappear from it so that the spleen is less richly supplied with lymphocytes than normally. Frequently the loss of the lymphoid elements leads to a disappearance of many of the follicular masses, leaving behind patches of endothelial cells. However, when this stage has arrived, evidences of endothelial degeneration are prominent.

Associated with the loss of lymphocytes from the malpighian bodies, the endothelial cells in their peripheral portion and in the pulp immediately contiguous to them, become more prominent. These cells are larger and more numerous than normal; and are arranged along the borders of the vascular spaces or are loosely attached to the neighboring reticulum. Some of the cells appear quite free and show no regular relation to the fixed structures of the part.

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be mistaken for protozoan parasites. Ring formations like those of malaria, and leishmania types with a false nucleolus are not uncommon, but a differential staining shows these structures as irregular nuclear masses arising through fragmentation. In the advanced stages of the disease the debris arising from the endothelial cells is much increased and can be traced as a zone in the periphery of the follicles.

These reactions of the spleen differ from those of the liver and kidney where the parenchymatous cells alone suffer necrosis while the endothelial cells, though showing fatty degeneration, are not found to suffer complete destruction. During the stages of hyperplasia and early degeneration of the endothelial cells in the spleen, these cells may show the accumulation of fatty substances in their cytoplasm. Up to the present we have not observed similar reactions in the spleen of guinea pigs inoculated with the blood of yellow fever cases. The lesion is quite distinctive from that seen in human cases of relapsing fever.

The reaction arising in the spleen suggests the influence of a toxic agent brought to it by the blood or diffused into its tissues by a virus which is harbored locally. The absence of any evidence of a cellular exudate distinguishes the lesion from infections by the common bacteria. Unfortunately our analyses did not include the examination of the bone marrow to observe whether similar reactions, as those described by Bunting,⁸ Jordan,⁹ and others, in the megakaryocytes of the bone marrow, had taken place.

The quantity of red blood cell phagocytosis carried on by the reticulo-endothelial system is never great, and does not play an important rôle in blood destruction in yellow fever.

The only reference which we have encountered which deals with the changes arising within the spleen in yellow fever is that of Turnbull whose observations are very similar to our own. Turnbull, however, was able to carry out his observations on only two cases of yellow fever, and has not had the opportunity of observing the successive changes which arise in the course of the disease. We have found that a careful examination of the spleen is frequently helpful in assisting in the pathologic diagnosis where yellow fever must at times be differentiated from other conditions giving rise to liver and kidney damage.

is made up of oil droplets surrounded by a capsule of fibrous tissue showing various grades of hyaline degeneration. Whereas the oil droplets may become partly displaced by the capsule, they never disappear completely, even as long as six to nine months after the injection.

As noted in previous papers, tissue changes about droplets of coal tar may be altered by the diet given an animal. For our largest series of experiments we have used the white rat. For a smaller series we have used monkeys and guinea pigs to corroborate our findings in the rat. The same character of tissue change occurs about droplets of mazola oil, paraffin oil and neat's-foot oil. There is some variation in regard to the degree of change produced by these different substances, but as a class the reaction is the same. The state of nutrition of the animal, especially as influenced by the vitamin content of the dietary, regulates not only the degree of the tissue change about these oils, as about coal tar, but also regulates the manner of change which may take place in these substances themselves. It became evident, therefore, that coal tar and paraffin oils, classed as mineral oils; mazola oil, classed as vegetable oil; and neat's-foot oil, classed as animal oil, act in the same manner on the tissues. Our work on coal tar indicates that this action must be the result of their ability to dissolve lipid substances from the tissues. It became of interest, therefore, to observe if the same reaction in the animal is brought about by different fractions and different mixtures of these lipid solvents in the animals fed the different dietaries.

By removing the fats from coal tar with ether, we obtain a black substance that has the gross appearance of soot. It is in fine particles. Making a suspension of this with normal salt solution we injected different quantities into the subcutaneous tissue of rats.

We have used rats for the greater part of our experiments, because this animal does well on a food ration which conforms basically to that of man. Then too, spontaneous tumors are uncommon in the rat and our strain of rats has been inbred over a long period of time, accurate data having been kept of these different strains as to their period of life cycle, fertility and weight curves.

Even on a rich vitamin A ration there is an extensive early complete hyalinization of the tissue about the particles of soot. This soot is evidently more active than crude coal tar because in a previous paper ² it was shown that coal tar produced a cellular reaction

LOCAL AND SYSTEMIC CHANGES INDUCED BY LIPOID SOLVENTS IN ANIMALS FED ON DIETS VARYING IN VITAMIN CONTENT *

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In this paper we will give additional data in regard to the local and systemic changes produced in the laboratory animal as a result of the introduction of any one of several lipid solvents into the subcutaneous tissue. We have indicated the nature of these local and systemic changes and their relation to different dietaries in a preliminary report.¹

The great majority of chemical substances that are now known to produce cancer are fat solvents, the same agent producing either carcinoma or sarcoma. That some general change in the organism as a whole regulates the degree of reaction induced about the lipid solvent in a localized area of tissue and the relationship of this to the formation of malignancy, has been emphasized in former papers from this laboratory.^{2,3} Wolbach and Howe⁴ have noted that diets deficient in vitamin A cause a transformation of the epithelial layer into stratified squamous keratinizing epithelium in the upper respiratory tract, salivary and accessory salivary glands, later in the pancreas, renal pelvis, bladder, seminal vesicles, epididymis and prostate gland.

In our animals fed a diet rich in vitamin A the local tissue change about the droplets of a lipid solvent is much different from that in the animals fed balanced vitamin dietaries and diets deficient in vitamin A. The variation of the vitamin B content has little significance except that adequate vitamin B must be present in the diet to balance the vitamin A or the animal will neither live for a long period of time nor have a normal growth curve. On the rich vitamin A diet the lipid solvent breaks up into numerous small droplets in the local area, and a zone of fibroblasts surrounds each one. There is a certain amount of proliferation as evidenced by the presence of mitoses for a period of thirty days or longer. A tumor persists that

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the ergusia of the cell. As pointed out in another paper¹ coal tar acts most intensely when first introduced. Its action then wanes as any substance that is being used up or is dissolving some fraction from the tissue. In proof that it dissolves a fraction from the tissue, it has been found that it can migrate from the initial point of inoculation after a given period of time. This ability to migrate is the same as noted in fat droplets taken from cells. Burrows⁹ found these fat droplets saturated with ergusia. He proved that they were so saturated by showing that they coagulate blood while the same fat as it is fed to animals prevents the coagulation. If the ergusia is the same as one or all of the fat soluble vitamins, then these vitamins should protect the animal against the local action of the lipid solvent.

The zone of tissue around droplets of mazola oil or coal tar undergoes hyaline degeneration after a certain period of time in the laboratory animal fed a stock ration of dog biscuits, lettuce, meat, corn and water. This hyalinization is similar to that seen in the tissue surrounding a malignant growth. Burrows' work indicates that such degeneration is the result of the removal of the ergusia from the tissue. A different set of conditions takes place when the mazola oil is not allowed to remain in the subcutaneous tissue for a long period after the injection, but is aspirated every two days and fresh oil injected after each aspiration. At the end of thirty days the resultant tumor is made up of cell nests containing many mitoses. The adjacent lymph nodes contain a few oil droplets. At a later period two sets of condition have taken place; there are areas of hyaline tissue and areas made up of densely packed cells. Some of these cell masses have invaded the oil droplet similar to the invasion noted by Burrows and Johnston⁹ when Allen-Doisy hormone is added to the mazola oil injected.

The differences in tissue change about these oil droplets in the animals on the various dietaries may be related to the clinical course of oil tumors in man. In different individuals, oil tumors undergo the same variation that we have found resulting from giving closely related animals different dietaries. The oil tumors in one individual may disappear or at least cause no trouble. In another individual they may persist or later spring into activity. That these changes are related to the nutrition of the patient is indicated by a recent case which came to the laboratory. This was a case with multiple

in rats fed a dietary rich in vitamin A. Hyaline changes occur quickly in animals fed a dietary deficient in vitamin A. From experiments with the tissue culture⁵ it has been shown that fats and oils act to remove the ergusia, a lipoid constituent of the cell. Later work^{6,7} has shown that the ergusia is either vitamin A or replaced by vitamin A. Burrows⁸ has further shown that the chemistry of cells and intercellular substances is such that the removal of the ergusia should cause either hyaline or granular changes. In terms of these analyses we have concluded that the coal tar soot is more active than coal tar in dissolving this fraction.

In other experiments we injected two cubic centimeters of cod liver oil, two of neat's-foot oil, two of mazola oil and two of paraffin oil, subcutaneously in a series of rats fed diets deficient in and rich in vitamin A respectively, with a series on a balanced vitamin dietary and a stock laboratory ration dietary as controls. In every instance the diet rich in vitamin A caused the oil to become broken into many smaller droplets. Around each droplet there is an increase in the number of fibroblasts and less degeneration. In the animals on the vitamin A deficient diet, the lipoid solvent remains in one large droplet and the tissue about it shows marked hyalinization. Coal tar causes the greatest degree of hyalinization, the tissue change about the paraffin, mazola and neat's-foot oil being less in degree but of the same type. In our studies with cod liver oil injected under the skin, we have found that it may form into a cheesy-like mass and does not in all cases become encapsulated like neat's-foot oil or oil extracted from rat tissue. Neat's-foot oil and cod liver oil are animal oils, but no doubt differ greatly in their content of vitamin A. There is no significant difference between the reaction about neat's-foot oil (animal), mazola (vegetable) and paraffin oil (mineral) in the subcutaneous tissue when they are made sterile before injection and introduced under sterile conditions.

Cod liver oil contains large quantities of fat soluble vitamins. In this regard it differs from the oils mentioned above. In this same regard it is interesting that tumors similar to those produced by other oils were seen about droplets of cod liver oil in only two cases out of twenty-five. The relation of this change of reaction to the high vitamin content of this oil is now being investigated.

These studies have given further evidence of the similarity of ergusia to vitamin A. At least they indicate that vitamin A supplies

the liver. The mixture of paraffin oil and coal tar acts more quickly than either of these oils alone, or other oils we have used. The interesting fact is that the different oils and mixtures of oils have each their own time interval between their injection and the appearance of the fatty liver. The mixture of coal tar and paraffin oil produces this change in 30 days after a single injection of two cubic centimeters, the same quantity of a mixture of coal tar and mazola oil produces this change after 40 to 60 days, mineral oil alone after 45 to 60 days, mazola oil alone after 60 to 90 days, and coal tar alone after 90 days. The growth curves of the rats in this series are interesting, in that they indicate the time of the appearance of fat in the liver. There is always a transient decline in weight preceding the appearance of fat in the liver.

The livers of these rats were studied not only grossly but microscopically. The liver of the rats fed on a high vitamin A diet and injected with the lipoid solvents contained very large quantities of fat which stained sharply with Sudan III. The fat was contained in large irregular vacuoles, which had smooth contours. The remainder of the liver cells were well preserved. These livers did not resemble in any way the necrotic fatty livers produced by injecting chloroform, phosphorus, etc.

To determine the relationship, if any, between the lesion we observed in the above series and that produced by chloroform, a series of rats were placed on rations containing different quantities of vitamin A, different ratios of vitamins A and B, and different ratios of carbohydrate, protein and fat. It has been well established by Opie and Alford¹³ and others, that the toxic effects of chloroform on the liver can be prevented to a certain degree by a high carbohydrate diet. In a part of our series the chloroform was added to a lipoid solvent (mazola oil, paraffin oil or neat's-foot oil) and the mixture injected into the subcutaneous tissue according to the technic used in the production of oil tumors described by us in former studies. In a part of the series the chloroform was administered by inhalation. The hepatic lesion in these animals is a necrosis associated at times with a small amount of fatty infiltration. The picture is different from that of the above series, the chloroform lesion being one of destruction, the coal tar, paraffin, mazola lesion being one of fatty infiltration in a cell with intact nucleus. In corroboration of the work of Opie and Alford and others, we found that a high

oil tumors in the sheath of the deltoid muscle.¹¹ These tumors resulted from an oil injection eleven years previous and had caused no trouble until she was placed on a vitamin-free, salt-free, dietary. She was placed on this dietary for the treatment of nephritis. On the salt-free, vitamin-free dietary, the oil tumors increased rapidly in size and became painful, only to recede again to their former size on a vitamin rich dietary. Microscopically these tumors are similar to the tumors produced by the introduction of lipid solvents into the tissue of the laboratory animal (rat, guinea pig and monkey). The oil has broken up into small droplets. We do not know the nature of this oil, but probably it is mineral or vegetable oil used as a menstruum for the administration of a drug. Each droplet is surrounded by a zone of cellular fibrous tissue containing a few lymphocytes, plasma cells and giant cells. From previous studies on animals this reaction in this patient can be explained as the result of the oil removing the ergusia from cells about it. So long as the individual was on a balanced vitamin dietary the tumor remained quiescent, because the ergusia absorbed by the oil was replenished by the system. When the vitamin A was withdrawn from the dietary an imbalance resulted, the tissue about the oil droplets became deficient in ergusia and as a result the cells were allowed to grow.

In a study of the gross and microscopic sections of the internal organs of the rats injected with these lipid solvents or mixtures of them, we noted a striking departure from the usual in the series fed on a diet rich in vitamin A. These rats always developed a fatty liver sooner or later. Visible fat in the liver has not been observed as the result of feeding a diet rich in vitamin A. It occurs in these animals only when they are also injected subcutaneously with sufficient doses of one or the other lipid solvents.

The amount of fat in the rich vitamin A diet is also evidently not concerned with the appearance of fat in the liver. The appearance of fat in the liver was associated with the high vitamin A content of the fat. Diets containing equal quantities of crisco in place of butter were not associated with fatty livers in animals receiving sufficient quantities of lipid solvents injected subcutaneously.

In our preliminary report¹² we stated that a mixture of paraffin oil and coal tar is peculiar in that it produces a fatty liver. Our later work has indicated that this is not true. Any lipid solvent injected in sufficient quantities subcutaneously will produce this change in

tissue or after inhalation of small amounts. The necrosis occurs even as soon as twenty hours after the inhalation or introduction of chloroform into the subcutaneous tissue. No doubt the whole body is saturated with the chloroform at this period of time. Inasmuch as the liver is presumably the site of highest concentration of ergusia in the body, much of the chloroform should be transported there early. Thus, the chloroform in the liver acts as coal tar in the subcutaneous tissue. Coal tar produces a certain amount of degeneration in the areas about it. Chloroform is a more drastic lipoid solvent. It removes more of the ergusia from the liver parenchyma with a resultant precipitation of the cell proteins and the typical picture of necrosis. With small doses of chloroform given to rats on a *protective* dietary, the lesion is periportal, it is around the blood vessels which no doubt carry the chloroform to the liver. If a *non-protective* dietary or larger quantities of chloroform are used, the whole liver undergoes necrosis, the animal dying as a result.

DISCUSSION

In previous experiments² it was found that coal tar produces both local and systemic changes when introduced into the tissue. It was shown also that the systemic change resembled grossly the cachexia of malignancy. These systemic and local changes vary with the vitamin content of the dietary given the animal, the carbohydrates, proteins, fats and salts being kept more or less constant. In these studies we have found that other vitamin free oils, animal and vegetable in nature, produce the same changes and respond in the same way to dietary.

In making these studies we have observed another interesting systemic change. There is an accumulation of fat in the livers of rats fed a ration high in vitamin A and injected with a lipoid solvent. We have not noted these changes in rats fed a ration high in vitamin A alone. The time of occurrence of this fatty infiltration varies with the oil or combination of oils used.

The results of the earlier experiments with coal tar were explained by assuming that the coal tar dissolved the ergusia from the tissues as Burrows had shown in the tissue culture. The fat soluble vitamins protected the animal against these effects by restoring the ergusia dissolved. These later observations can be explained as the

carbohydrate diet has a protective action against chloroform necrosis of the liver. A diet rich in vitamin A, the carbohydrate being low or moderate in amount, has no influence on these toxic effects. However, a diet rich in both carbohydrate and vitamin A protects the rat to a greater degree than a diet high in carbohydrate and not rich in vitamin A.

Since the livers from the rats injected with the lipoid solvent and given the high vitamin A diet show no destructive lesions in the liver microscopically, the presence of fat must be due to an accumulation of fats from the blood stream. The body as an organism may be considered as essentially an aqueous solution containing proteins, fats, carbohydrates and salts. As Burrows has demonstrated with the tissue culture¹⁰ the process governing the migration of fat droplets is not different fundamentally from that governing the migration of body cells. A droplet of fat will migrate from an area containing less to an area containing more ergusia. The droplet will remain stationary when it is saturated to the same degree as the environment about it. That the liver should become saturated with fat droplets in an animal fed a ration rich in vitamin A and not in an animal fed a dietary deficient in vitamin A when a lipoid solvent has been introduced locally into either of such animals is readily understood when we consider the migration of fat to be such a simple process.

Because the lesion in the liver produced by chloroform is that of necrosis and that produced by mazola, paraffin and coal tar is one of infiltration, it does not mean that these substances are not alike in their action. Coal tar, mazola oil and paraffin oil are not absorbable, they remain probably indefinitely, in part at least, in the area of tissue into which they have been injected. They remain as encapsulated tumor masses. In this local area they absorb slowly the ergusia from the tissues for a long time. In a system saturated with ergusia they cause enough disturbance of the ergusia balance so that the liver becomes filled with fat. The liver is much richer in ergusia than the subcutaneous tissue of the body, and especially the subcutaneous tissue from which a certain amount of ergusia has been removed by the lipoid solvent.

Chloroform is readily absorbed and is a more active lipoid solvent than the mineral, vegetable or animal oils. The necrosis of the liver occurs after introduction of the chloroform into the subcutaneous

changes are caused by some injurious substance in the cod liver oil. Höjer¹⁷ records experiments on rats proving that they are not produced by a poison in the oil, but are produced by an overdose of cod liver oil without simultaneously increasing the vitamin B in the food. We have noted no changes in organs other than the liver.

Plimmer and Rosedale¹⁸ first drew attention to the fact that the ill effects of large doses of cod liver oil could be overcome by increasing the quantity of vitamin B in the food. Their experiments were done with chicks. Studies in this laboratory show quite clearly that the same is true of overdoses of vitamin B. The ill effects of an overdose of vitamin B, whether it is given in the form of a commercial product, such as Marmite (vegex), autolyzed yeast, X-rays¹⁹ or young bacterial cultures,⁷ may be prevented to a certain extent by the addition of adequate vitamin A in the dietary. It is when there is a proper balance between these two vitamins that a functioning mechanism results.²⁰

Levine and Smith²¹ in a recent report state that the livers from rats fed a dietary containing as high as 86 per cent of the total calories in the form of fat, provided the protein and salt requirements of the animal are fulfilled, contain no fat droplets when examined microscopically.

CONCLUSIONS

1. Various oils and fats act like coal tar when injected into the subcutaneous tissue of animals. All of them dissolve the lipoids of the cell and may be classed as lipid solvents.
2. The cellular activity about droplets of various lipid solvents injected into the subcutaneous tissue of rats is greatly increased by feeding a diet rich in vitamin A.
3. The lipid solvent remains *en masse* in an animal fed a ration deficient in vitamin A and disperses into smaller droplets in the animal fed a ration rich in vitamin A.
4. The tissue reaction about the lipid solvent is altered in degree if the fats are removed from the solvent with ether before it is injected into the tissue.
5. Rats injected subcutaneously with certain lipid solvents or mixtures of them and fed a diet rich in vitamin A suffer a massive fatty infiltration of the liver. This infiltration occurs at different periods of time after the injection of the different lipid solvents.

result of a migration of fat from the tissues to the liver. This migration results because in the animals fed a high vitamin A diet the liver must be rich in these vitamins. The tissues on the other hand are depleted of them by the lipid solvent injected.

When vitamin A is added to the system the lipid solvent remains active in the subcutaneous tissue for a long period of time. Fibrous tissue forms around it, the ergusia and archusia in the area become balanced and hyalinization is prevented, due to the fact that the animal supplies the ergusia which is being removed by the lipid solvent. The animal is able to supply this ergusia because it is getting a large amount of vitamin A in its dietary. The lipid solvent absorbs ergusia until it is saturated, then breaks up into droplets and migrates to other organs of the body. We have noted droplets of lipid solvent in the lungs, in the liver and in the tissues a considerable distance from the oil tumor.

The variation in the amount of hyalinization in the tissue about the droplets of lipid solvent is a factor regulated apparently by the relative amount of ergusia and archusia in the tissue. In earlier studies² the variation of this factor was noted. There is an increased hyalinization of tissue about droplets of coal tar in the subcutaneous tissue of an animal fed a ration high in vitamin B. This is a system rich in archusia. This hyalinization was not so marked as that occurring about droplets of coal tar in an animal on a dietary deficient in vitamin A. This is a system low in ergusia and consequently high in archusia. The type of hyalinization is similar to that seen in areas surrounding malignant growths in man. Former studies of Burrows and Jorstad⁷ have shown that malignant tissue is very high in archusia. This area high in archusia is depleting the surrounding tissue of its ergusia with the resultant hyalinization taking place.

Considerable data have been published recently in regard to the availability of the vitamin A in the system, especially as influenced by the intake of vitamin B, or at least the concentration of vitamin B in the system. Collazo and Funk¹⁴ state that one cubic centimeter of autolyzed yeast permits the highest food intake on a diet with the ratio of carbohydrate to protein 8:1. This ratio corresponds to that found in cereal grains. Agduhr and Höjer¹⁵ have recently shown that an excess of cod liver oil in the dietary may cause ill effects. They find vacuolar degeneration and brown atrophy of the heart muscle in these cases. Agduhr¹⁶ concludes that these

6. Chloroform produces a necrosis of the liver because it diffuses rapidly throughout the system. This lesion is similar to that produced locally by coal tar in the tissue.

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creases, and new vessels become visible. The first difference between auto- and homoiotransplants to appear consists in an increase in the number of fibroblasts and lymphocytes in the homoiotransplants. In the latter, between the seventh and twelfth day, the fibrous tissue invasion becomes very intense in typical cases. Thus, individual acini and groups of acini are surrounded and destroyed by fibrous bands. The difference between auto- and homoiotransplants is especially marked in the centers of the transplanted pieces, which, in cases of the latter kind of transplant, are organized by dense connective tissue in which the vascularization is poorer than in autotransplants. In the homoiotransplants, lymphocytes are present in large numbers; by direct invasion and compression they destroy the acini and, having penetrated their lumen, destroy the colloid as well. On the other hand, wherever the thyroid tissue has not perished from the action of connective tissue or lymphoid elements, it is as well preserved as in the autotransplant. It may therefore be assumed that the destruction of the homoiotransplant is largely due to the action of lymphocytes and fibroblasts. In the majority of cases the thyroid is attacked with so great an intensity that at the end of twenty days there is little or no thyroid tissue left at this time, although the date of complete destruction varies in individual cases. The fate of the average homoiotransplant of thyroid gland in adult hosts is, therefore, a gradual disappearance of the gland tissue, and its replacement by connective tissue.

By varying the ages of the hosts in different experiments, we wished to determine in what manner the age of the host affects the character of the homoio-reaction just described. The effects of the age factor upon the degree of preservation of thyroid acini, on the colloid and on the intensity of lymphocytic and connective tissue reactions, were particularly studied. As stated above, in some cases, thyroid lobes were transplanted from the same donor simultaneously into mother and offspring. In such cases, the transplant into the mother served as control for the lobes transplanted into the young guinea pig. In other instances, pieces of thyroid transplanted into other adult animals were used as controls. In our first series, transplants into 20 adults and 20 young animals, and in the second, transplants into 34 adults and 25 young animals were examined; a total of 99 transplants, of which 54 were from adult, and 45 were from young hosts. On the whole, the results showed a better degree of

THE EFFECT OF AGE OF THE HOST ON THE FATE OF TRANSPLANTS OF THYROID GLANDS IN GUINEA PIGS *

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In these experiments, an attempt was made to determine what effect, if any, the age of the host, as exemplified by guinea pigs, had upon the reaction, towards homoiotransplanted thyroid gland. Male adult animals, weighing 500 to 700 gm. were used as donors. In some instances, the two lobes of thyroid were transplanted simultaneously, one into a female guinea pig and the other into one of the litter. In the majority of cases, the adult hosts were females, 500 to 800 gm. in weight, while the young, the age of which varied between four days and three weeks, and which weighed on the average 150 gm. were of either sex.

The technic used in these experiments was the same as that employed in previous experiments of this series of transplantations. The lobes of the thyroid were placed in subcutaneous pockets made in the abdominal wall. Two sets of experiments were carried out. In one, the transplants were removed for examination at intervals varying between ten and twenty* days after transplantation, while in the other series the interval was twenty days. The former experiments had already been done by Dr. Leo Loeb¹ and have been referred to by him in a previous publication.

The typical reaction to homoiotransplants in general has been described in the papers of Loeb and his associates² and Hesselberg,³ especially, as far as the thyroid gland is concerned. In the first four or five days after transplantation there is practically no difference in the reaction to auto- and to homoiotransplantation. During the first day or two, large central parts of the gland become necrotic. After forty-eight hours, the first fibroblasts, a few polymorphonuclear leucocytes and lymphocytes surround the graft, and appear in the center of the transplant in seventy-two hours. In four days, regeneration of acini begins, the necrotic center becomes smaller, the number of fibroblasts and lymphocytes in the necrotic part in-

* Received for publication June 6, 1927.

these latter transplants there was a marked lymphocytic reaction, whereas in the other two, the number of lymphocytes was small. At the same time a large quantity of dense connective tissue had been formed in which only isolated islands of thyroid tissue were left. One transplant of fifty days still showed small acini with colloid and an infiltration by a dense mass of lymphocytes.

As an example of typical findings after transplantation of thyroid gland into adult hosts we cite the following protocol of an experiment:

Experiment I. Guinea pig No. 209; weight 560 gm. Transplant removed for examination twenty days after operation. The piece is well encapsulated by fibrous tissue that shows numerous connective tissue cells among the regularly arranged collagen fibers. The peripheral capsule contains large numbers of lymphocytes that accumulate in groups, and appear especially within the capsule and along its internal border. Along the outer margin of the capsule and occasionally enclosed within it, are seen some fat cells. The center of the piece is well organized by connective tissue containing relatively few fibroblasts, a few capillaries and very few lymphocytes; both lymphocytes and connective tissue cells are more numerous toward the periphery and less numerous toward the center. The blood vessels pierce the capsule, enter from the periphery, branch and penetrate toward the center. Clusters of cells scattered toward the periphery consist essentially of collapsed acini or parts of acini, none of which contains colloid, and epithelial cells occurring singly or in isolated groups. These are surrounded by lymphocytes that are wedged between adjacent acini. The thyroid cells are large and vesicular, round or oval. In the interstices between these islands of cells and elsewhere, the transplant is organized by fibrous tissue. (Fig. 1.)

While the preceding record represents the typical finding in the case of transplantation into adult guinea pigs, the following is the protocol of an infrequent case in which the preservation in the adult host is much better than in the average experiments.

Experiment VII. Guinea pig No. 53; weight 695 gm. Transplant removed after twenty days. Weight of animal at end of experiment, 745 gm. A thick, fibrous capsule encloses the tissue. The preservation of thyroid in this case is unusually good; large and small acini, filled with colloid, being numerous. The largest acini are situated

preservation of thyroid tissue in young animals than in the adult hosts, though the longer the transplant remained in the host, whether old or young, the greater was the tendency towards destruction of the glandular tissue.

We shall consider first the fate of transplantations into adults, in both series. In the first series, we found seventeen pieces of thyroid tissue, or 85 per cent of the transplants into adults, showing little or no preservation. Included among these cases were eight pieces which showed poorer preservation than those from corresponding young animals, two pieces in which destruction was complete in both old and young and seven cases of practically an entire disappearance of the thyroid gland in the adult, while in the average of the young hosts, which served for comparison, the preservation was better. In these transplants into adult guinea pigs, fibrous tissue and lymphocytes were much in evidence, though an occasional acinus or peripheral group of acini containing colloid, could be found. However, there were three transplants into adult hosts in which the thyroid tissue was relatively well preserved. One, a fourteen day transplant, showed so little reaction that it resembled an autotransplant. In the other two, which were likewise fourteen day transplants, the number of preserved acini was smaller, though in the periphery of the transplant they were more numerous and larger than those usually found in this kind of homoiotransplantation. However, a rather marked lymphocytic and connective tissue reaction was noticeable which separated and destroyed the glandular elements.

In the second series, the twenty day transplants, twenty-seven or almost 80 per cent of the transplants into adults showed practically a complete destruction of the thyroid gland, with replacement by dense fibrous tissue and with a greater or less degree of lymphocytic infiltration. In some instances an isolated, collapsed or fragmented acinus, free from colloid, was present. Of the remaining twenty day transplants, one (Fig. 5) showed a peripheral ring of well preserved acini, containing deeply stained colloid, with only a comparatively mild lymphocytic and fibroblastic reaction. This picture strikingly resembles a syngenesiotransplant. Another showed a fairly well developed ring of preserved acini containing colloid with an extremely severe lymphocytic reaction. In the other four, a few acini were found varying greatly in size and in quantity of colloid; in two of

the adult control shows almost complete replacement by fibrous tissue. On the other hand, in eleven cases, or 44 per cent of the animals included in this series, the picture differs little, if at all, from that found in about 80 per cent of the transplantations in the adult host. In fact, in a few instances, the degree of scarring is much more marked in the young than in the adult controls. (Fig. 7.) There are a number of transplants omitted from consideration because it is quite evident on examination that infection has taken place and destroyed the thyroid with replacement by connective tissue. In these cases, accidental factors vitiate the result. In another instance, the transplant appears to be entirely hemorrhagic, without showing the usual lymphocytic reaction, though organization of the hemorrhagic area has begun. (See Table I.)

TABLE I

Showing the frequency of thyroid preservation

Degree of preservation	20 Day Transplant		10-20 Day Transplant	
	Adult Guinea Pig	Young Guinea Pig	Adult Guinea Pig	Young Guinea Pig
Good.....	2 (6%)	13 (52%)	1 (5%)	15 (75%)
Medium.....	5 (14%)	1 (4%)	2 (10%)	0
Poor.....	27 (80%)	11 (44%)	17 (85%)	5 (25%)
Total.....	34	25	20	20

In analyzing the results of these transplantations, we compared the intensity of the lymphocytic and connective tissue reactions in the young and adult hosts. In the cases in which the preservation of the tissue in the young is not good, we could distinguish three groups of transplants: (1) In the first group there was a more marked lymphocytic reaction and a less dense connective tissue formation in the young than in the adult host. (2) In the second group the intensities of reaction in both were equal. (3) In the third group, in the adult, the reaction was typical, whereas in the young the transplant had been replaced by a scar without noticeable lymphocytic reaction. However, the number of transplants from young hosts belonging to these three groups was relatively small. In general, if we consider only those transplants in which very little or no tissue is

toward the periphery. In the center the acini have been replaced by loose connective tissue in which some fibroblasts and lymphocytes are seen. Lymphocytic infiltration is comparatively slight throughout. Many lymphocytes are found within the acini, partaking in the destruction of the colloid, which in places is retracted or shows vacuolization. The acini vary in shape, apparently in consequence of compression by the surrounding invading cells which in places separate the acini widely. The connective tissue reaction is not marked, though the center shows signs of organization. Blood vessels also enter the central part. At one pole, a rather dense fibrous band isolates an island of acinar tissue from the rest of the gland. (Fig. 5.) The picture of this transplant so resembles that found in the case of syngenesiotransplantation as to suggest that the donor and host in this experiment were related.

Considering next the fate of transplantations into young hosts in both series, we may state that it is more common to find a more or less complete peripheral ring of well preserved, large, colloid containing acini still present at the end of twenty days. In the first series, in which the pieces were removed ten to twenty days after transplantation, fifteen pieces, or 75 per cent of the transplants, show such a preservation, whereas of the remaining five, one, a twelve day transplant, has only a few acini and they are free from colloid; another, a sixteen day transplant, has only a few acini but all of these contain colloid. In another experiment in which only scar tissue is found, the possibility of infection must be considered. Of the remaining two transplants, one shows a somewhat better preservation than is found in the corresponding adult host; while in the other, a severe reaction on the part of the connective tissue, with the formation of fibrous tissue similar to the reactions in the adult controls, has taken place.

In the second series, in which examination took place after twenty days, fourteen cases, or 56 per cent of the transplants, show a good preservation of acinar tissue. Rather sharply contrasted with the practically complete destruction of thyroid observed in typical instances of transplantation into adult hosts which served as controls, the transplants into the young show, on the whole, a better preservation than is found in the best cases in the adult hosts. In one instance in which preservation in the young might be termed as only fair, merely a limited number of small acini being left, the piece from

denced by the presence of vacuoles in the colloid and numerous lymphocytes in the acinar lumen. More peripherally there are large, uninjured acini, with retracted and normal-appearing colloid. The entire transplant is surrounded by a fibrous capsule. (Fig. 3.)

As an example of the less frequent case of complete destruction of the transplant in a young host, the following protocol is presented:

Experiment XLII. Guinea pig No. 456; weight 158 gm. Age less than three weeks. Transplant removed in twenty days. The picture is one of complete replacement of the transplant by connective tissue. A peripheral capsule of young fibroblasts surrounds a denser central area. In the center are a number of typical epithelial pearls, the centers of which are completely hyalinized, whereas their peri-

TABLE II

*Showing the Intensity of Lymphocytic and Connective Tissue Reactions *
Twenty Day Transplants*

	Lymphocytic reactions	Connective tissue reactions
Stronger in adult hosts.	5	11
Stronger in young hosts.	15	4
Equal in adult and young hosts.	2	4

* This table includes transplants with and without preservation.

phery is composed of squamous epithelium. Very sparse scatterings of lymphocytes are seen. No thyroid tissue is found anywhere. (Fig. 7.)

The twenty day transplants are tabulated according to the relative degrees of lymphocytic and connective tissue reaction. The figures show, in general, that the connective tissue reaction is stronger in the adult hosts, whereas in the younger hosts, at the end of the twenty day period, the lymphocytes are more numerous.

In agreement with the classification of Loeb,¹ we may distinguish three classes of transplants according to the severity of the reaction on the part of the host. In Type I, no thyroid is left; there is merely a replacement of the transplant by connective tissue and a few lymphocytes (Figs. 1 and 7). In our experiments, most of our transplantations into adults fall into this class. Type II shows only a few

preserved, there is little difference in the intensity of the lymphocytic and connective tissue reactions in transplants from young and adult hosts. Indeed, there was, perhaps, a greater density of the connective tissue in the young. However, if we compare the transplants from the young, which show a relatively good preservation, with the typical transplants from adult hosts, we find the connective tissue reaction less marked in the young than in the adult, whereas the lymphocytic reaction, on the contrary, in such cases is much more marked in the young than in the adult hosts. It can readily be understood that the thyroid tissue which remains in the young, being greater in amount, is able to attract a larger number of lymphocytes than the few scattered acini which survive in the adult hosts.

The following protocol may serve as an example of a typical transplantation in the young, in which the preservation of the tissue is relatively good:

Experiment I. Guinea pig No. 235; weight 125 gm. Age less than three weeks. Transplant removed after twenty days. This young guinea pig, and the corresponding adult guinea pig No. 209 (Fig. 1) each received one lobe of thyroid from the same donor, the transplant in the adult serving, therefore, as a control for the tissue obtained from guinea pig No. 235. There is a very thin capsule surrounding the transplant in the young animal. In the periphery there is a ring of acini containing colloid, the acini being largest towards the periphery, while the smaller follicles are more centrally located. A large vessel enters from the periphery, giving off branches along its course. Blood and lymph capillaries seem to be most numerous in the center where organization of the transplant by connective tissue is most complete. In many places, lymph vessels, filled and surrounded by lymphocytes, may be seen. In the central, dense, fibrous tissue there are few cells. Strands of collagen fibrils ramify throughout the transplant. In a middle zone between the center and the peripheral acinar ring, many lymphocytes, but no normal acini are seen. However, there may be found an occasional, collapsed acinus surrounded by lymphocytes. In general, connective tissue and lymphocytes are abundant in the transplant. The peripheral ring of acini is interrupted in places, the acini here being replaced by masses of lymphocytes. In other places, individual lymphocytes invade the acini, enter between the acinar epithelial cells, appear in the colloid and subsequently destroy the latter, as evi-

DISCUSSION

In typical homoiotransplantations, the connective tissue reaction is usually completed in a period of from seven to twelve days following the operation, while the lymphocytes, the presence of which depends upon the formation of homoiotoxins induced by the difference in the individuality differentials in host and transplant, are usually few or lacking at this time. They usually appear somewhat later, the time of appearance depending on the difference between the individuality differentials. The greater this difference, the sooner the lymphocytes appear and the more numerous they are at a certain time. However, the intensity of the lymphocytic reaction must also depend upon the size and functional efficiency of the living portion of the transplanted tissue after a given period. If, as a result of accidental conditions or a very marked degree of incompatibility between the individuality differentials, only small amounts of tissues are left, the lymphocytic reaction may be relatively slight. In the typical homoiotransplantations we find usually a combination of marked connective tissue and lymphocytic reaction in and around the transplant in adult hosts, but this is found in only about 35 per cent of all transplants into young animals. In the majority of the latter, although the lymphocytic reaction is present, it is very intense only in the twenty day pieces, whereas the connective tissue reaction seems to be absent or very slight in these cases. The characteristic infiltration of homoiotransplants by connective tissue fails to appear in the young hosts in a large proportion of cases which seems largely responsible for the preservation of so much glandular tissue. Instead of a dense central fibrosis with interlacing connective tissue bands extending throughout the specimen, there is usually found, after transplantation into young animals, only a small central plug of fibrous tissue surrounded perhaps by some loose edematous connective tissue and scattered bands of connective tissue. This condition exists in a large number of transplants into young hosts. No dense fibrous tissue surrounds or compresses the acini, but the destruction of the latter, when it occurs, is accomplished largely by the lymphocytes which appear in dense masses in response to the large quantity of preserved glandular tissue. There is thus a great similarity between the character of these transplants and the moderately well preserved tissue of syngenesiotransplants. In both, the

groups of acini left within dense fibrous tissue. The center of the piece consists of dense connective tissue; lymphocytes are numerous, and most of the acini are collapsed and without colloid. Some small acini do contain soft colloid or desquamated epithelial cells. There are perhaps three transplants into adults which can be included in this group. In Type III, we find, in certain parts of the transplant, conditions similar to those characteristic of Type II; there is marked fibrosis, the acini are collapsed and free of colloid; lymphocytic infiltration is dense, but other parts show groups of acini which are close together, without fibrous tissue intervening between them. There may be medium and even large-sized acini in the periphery, with colloid in the process of absorption. Lymphocytes surround the acini, and act apparently as agents of destruction. Of this type there are four cases among the transplantations into adults. Such results are occasionally found in any fairly large series of homoio-transplantations. They seem to be due to a similarity of the individuality differentials of hosts and donors, which should be expected in some cases in accordance with the law of probability. Such similarity would tend to diminish the intensity of the lymphocytic infiltration or to delay its appearance, consequently delaying the rapidity of destruction of the thyroid transplant. Had the transplant been left in the host for a longer period of time, as for instance, forty or fifty days, it is not unlikely that it would have been entirely destroyed, since Type III is not commonly found at so late a date. In addition, we observed two or three cases in which preservation was even better than that which corresponds to Type III. There was only a mild connective tissue reaction. The center consisted of loose connective tissue and few lymphocytes. A complete peripheral ring of well preserved acini was present, the latter lying close together and containing good colloid. (Fig. 5.) This picture corresponds to that described by Loeb⁴ as Grade V and as the reaction characteristic of a syngenesiotransplant. We may assume that in all probability in such cases there has been a relationship between the donors and the hosts and that we have therefore to deal in reality not with homoiotransplants but with syngenesiotransplants.

greater intensity of the reactions on the part of the connective tissue of the host and under certain conditions also of the lymphocytes, there may be a greater direct injurious action of the body fluids of the older host on the transplant. However, since the injurious reactions on the part of the host tissues against the transplants are so marked in the older animals, it is difficult to prove that the latter factor plays a significant rôle in the destruction of the homoio-transplant.

As to the mechanism underlying the greater intensity of the reaction on the part of the adult host, we may assume that the individuality differential is as fully developed in the young as in the older host, but that the latter has developed mechanisms which insure a more marked reaction on the part of the host against the strange transplant, whereas in the young hosts these mechanisms are still in a more rudimentary state. Thus in the young hosts often a syngenesio-reaction is obtained instead of the typical homoio-reaction of the adult host.

SUMMARY

If we compare the reactions of adult with those of young guinea pigs against homoiotransplanted thyroid gland we find a more pronounced average reaction on the part of the adult hosts. This reaction manifests itself principally through the early and intense ingrowth and fibrous transformation of connective tissue cells, which exert a destructive effect on the transplant. There may, in addition, be a marked lymphocytic reaction in either host but, in the adult host as a result of the early destruction of great parts of the transplants, the remaining tissue cannot subsequently produce a quantity of homoio-toxins sufficient to attract large masses of lymphocytes around and in the transplant. Now, in the young host the transplanted tissue is still preserved in larger amount and is therefore functionally more active; in this case, the lymphocytic reaction may at a somewhat later date following transplantation be more intense in the young than in the adult host. Homoiotransplants in the young may thus resemble in certain cases syngenesiotransplants in the adult.

characteristic features consist of a very slight connective tissue reaction combined with delayed lymphocytic infiltration. In their various series of auto-, syngenesio-, homoio- and heterotransplantations, Loeb ^{4, 5 and 6} and his collaborators, especially Myer,⁷ Hesselberg³ and Sale,⁸ have shown that in general, a definite and early connective tissue reaction indicates a marked divergence of the individuality differentials of host and donor, whereas a delayed lymphocytic reaction signifies less divergence.

It is generally assumed, though apparently without definite experimental basis, that in young individuals, tissue transplants find a better soil than in older individuals. In the case of tumor transplantations, the number of takes seem to be greater in younger than in older individuals; at least this is true in the case of certain transplantable tumors. However, the conditions existing in tumor transplantations are not in every respect the same as those in ordinary tissue transplantations. In this connection it may be of interest to point to the experiments of Murphy⁹ who found that heterotransplantation of tumors and also of tissues may succeed, at least temporarily, in the allantois of chick embryos but simultaneous transplantation of spleen tissue causes a reaction to take place against the transplant. In our experiments we compared the fate of transplants of ordinary tissue, like thyroid, in young hosts who have passed the nursing stage, and in adult hosts of the same species, and we found distinct differences in the reactions between younger and older hosts. In the latter it is especially the connective tissue reaction on the part of the host which is more pronounced than in the younger hosts; while the lymphocytic reaction may at a certain period be even more intense in the young hosts, owing apparently to the fact that in the young the lessened intensity of the connective tissue reaction, or its absence, permits the preservation of a greater part of the transplanted tissue, which thus is able to call forth the lymphocytic reaction. When enough tissue has been preserved in the older host, the lymphocytic reaction also may be quite pronounced. This separation between the connective tissue and lymphocytic reaction is in agreement with the earlier observation of Loeb on the behavior of syngenesio- and homoiotransplants. In the former, he found the connective tissue reaction to be relatively mild or lacking altogether, whereas the lymphocytic reaction may become very intense at a later date. It is possible that in addition to the

DESCRIPTION OF PLATE

PLATE 128

FIG. 1. Experiment I, Guinea Pig No. 209. Low magnification showing the typical reaction to homoiotransplanted thyroid gland in adult animals after twenty days.

FIG. 2. Higher magnification of the peripheral part of the section shown in Fig. 1.

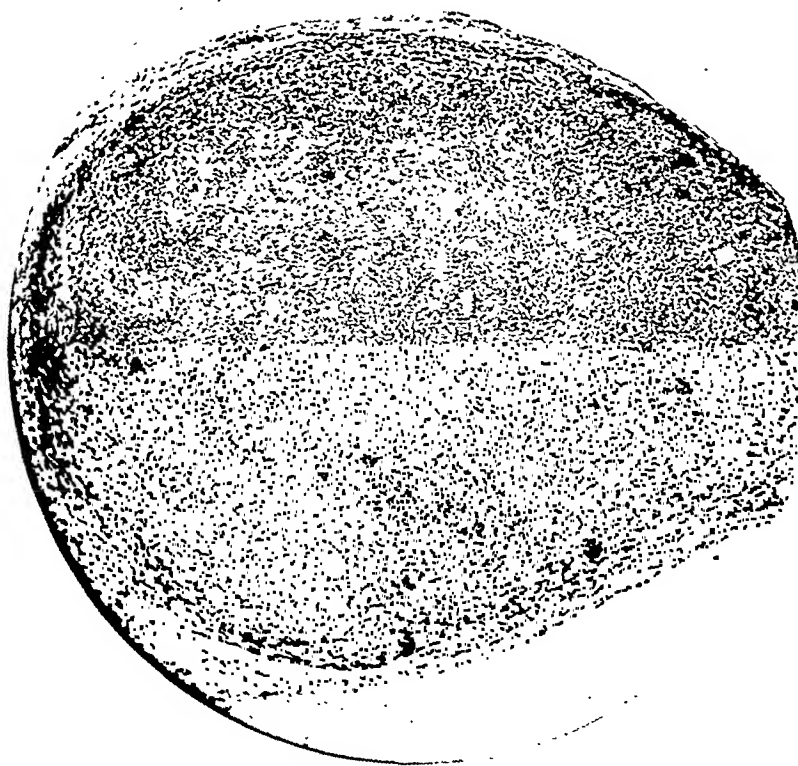
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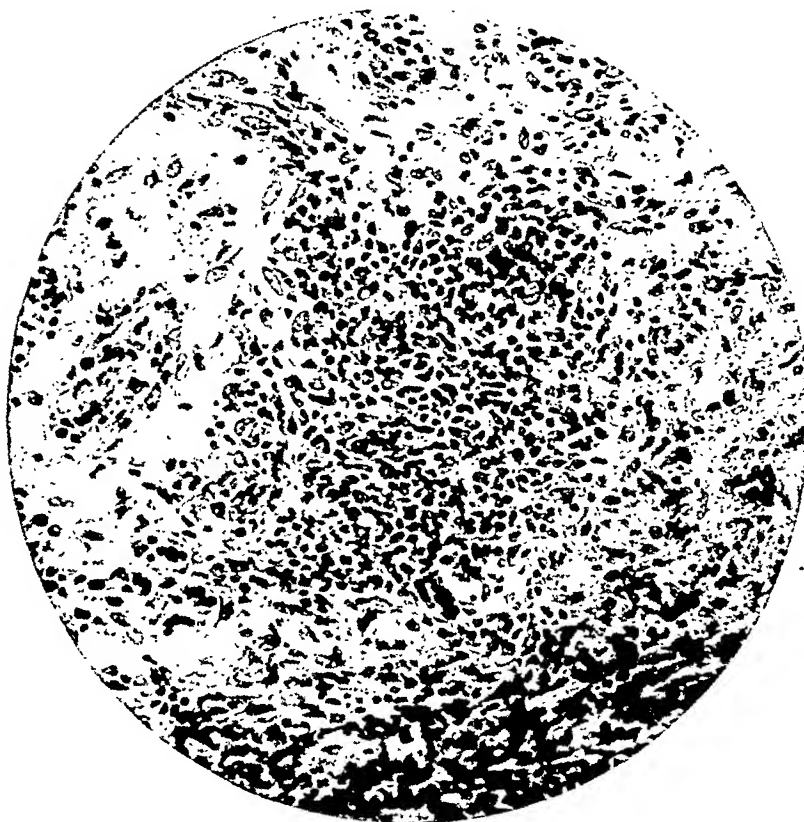
PLATE 129

FIG. 3. Experiment I, Guinea Pig No. 235. Low magnification, showing preservation of a peripheral ring of acini in homoiotransplanted thyroid gland in a young animal after twenty days. Both transplants shown in Figs. 1 and 3 were taken from the same donor.

FIG. 4. Higher magnification of the peripheral part of Fig. 3.



1



2

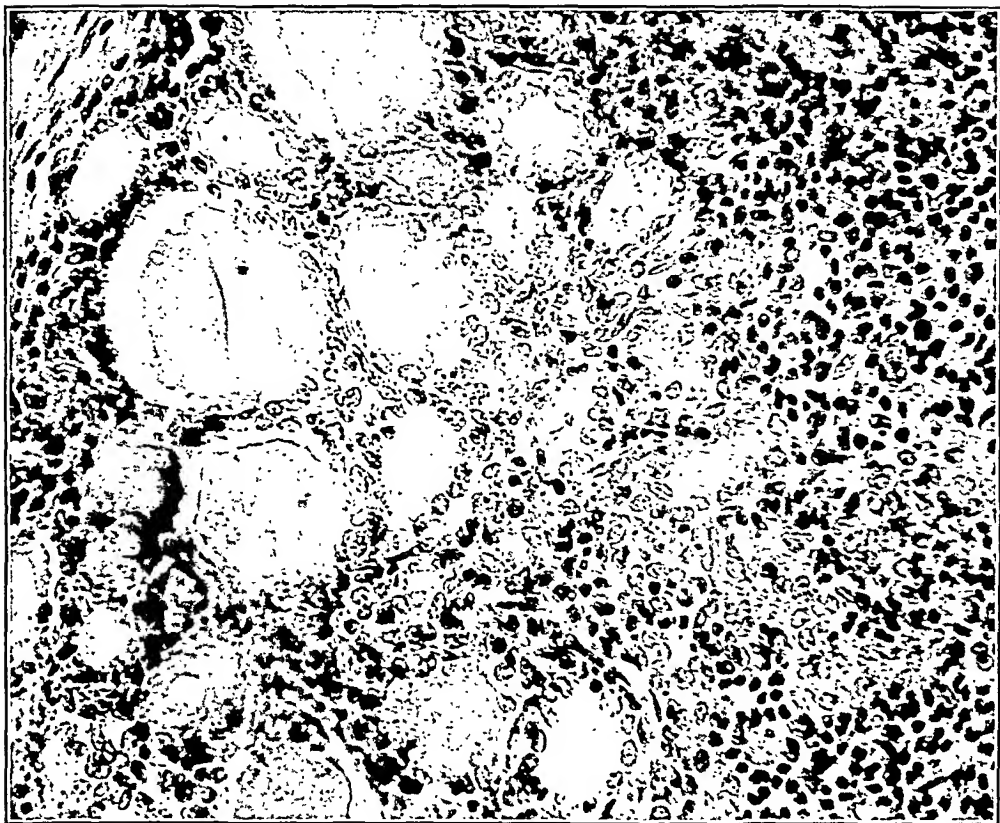
PLATE 130

FIG. 5. Experiment VII, Guinea Pig No. 53. Homoiotransplantation to adult animal. This figure shows a degree of preservation of thyroid tissue that is better than that found in the average transplantation into adults after twenty days.

FIG. 6. Higher magnification of Fig. 5.



3



4

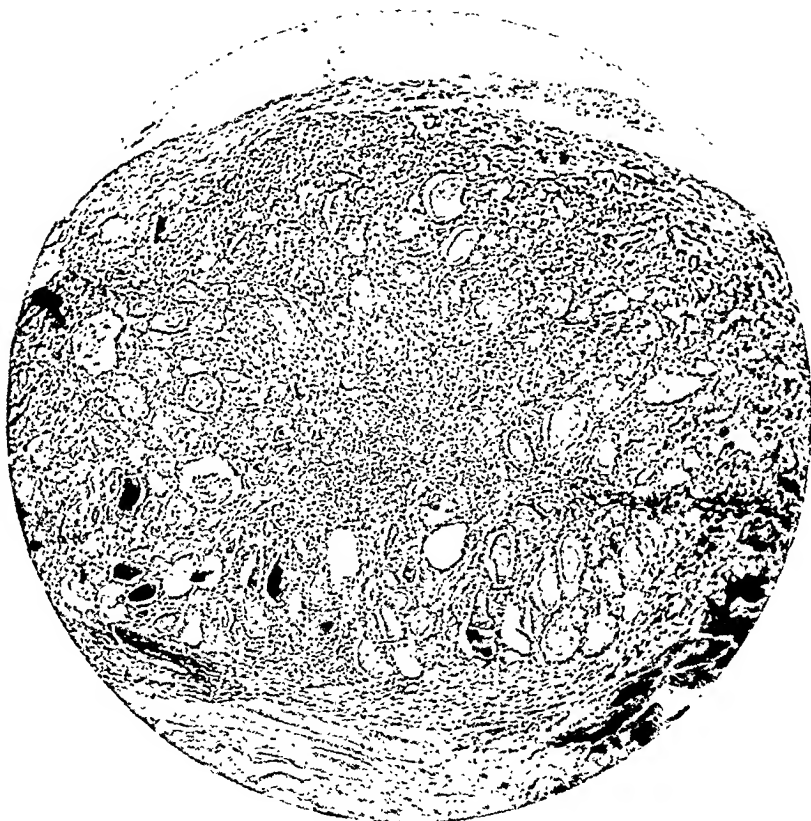
Tureen

Transplants of Thyroid Glands in Guinea Pigs

PLATE 131

FIG. 7. Experiment XLII, Guinea Pig No. 456. Section through thyroid homoiotransplanted to young animal. This photomicrograph shows an unusually severe connective tissue reaction with complete destruction of the transplanted thyroid tissue at the end of twenty days.

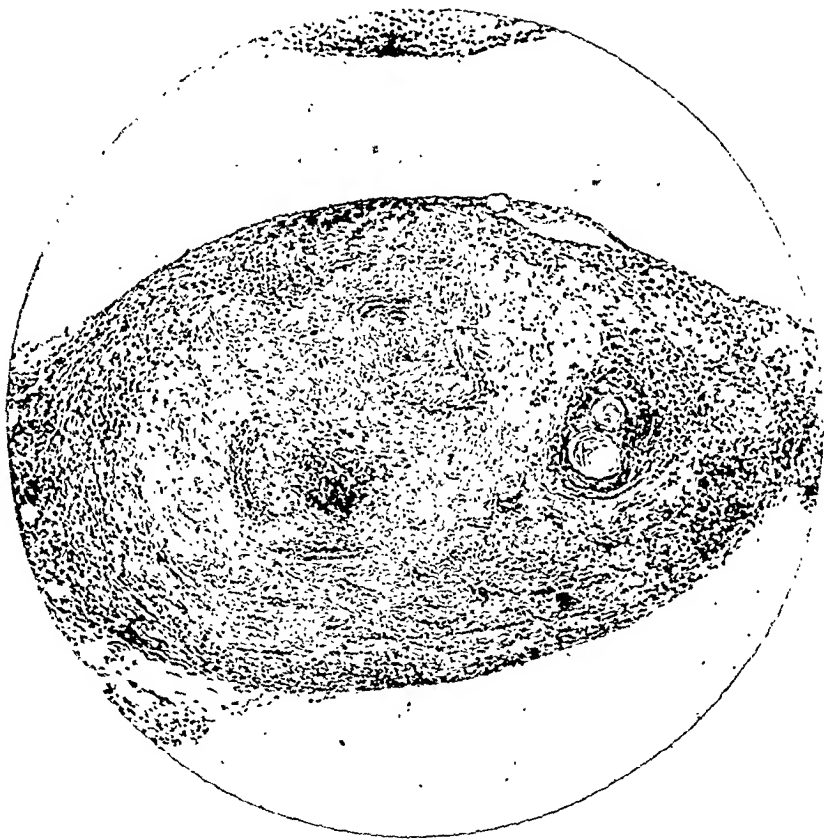
FIG. 8. Experiment XXV, Guinea Pig No. 223. Showing peripheral preservation of glandular tissue after twenty days; homoiotransplantation of thyroid into young animal.



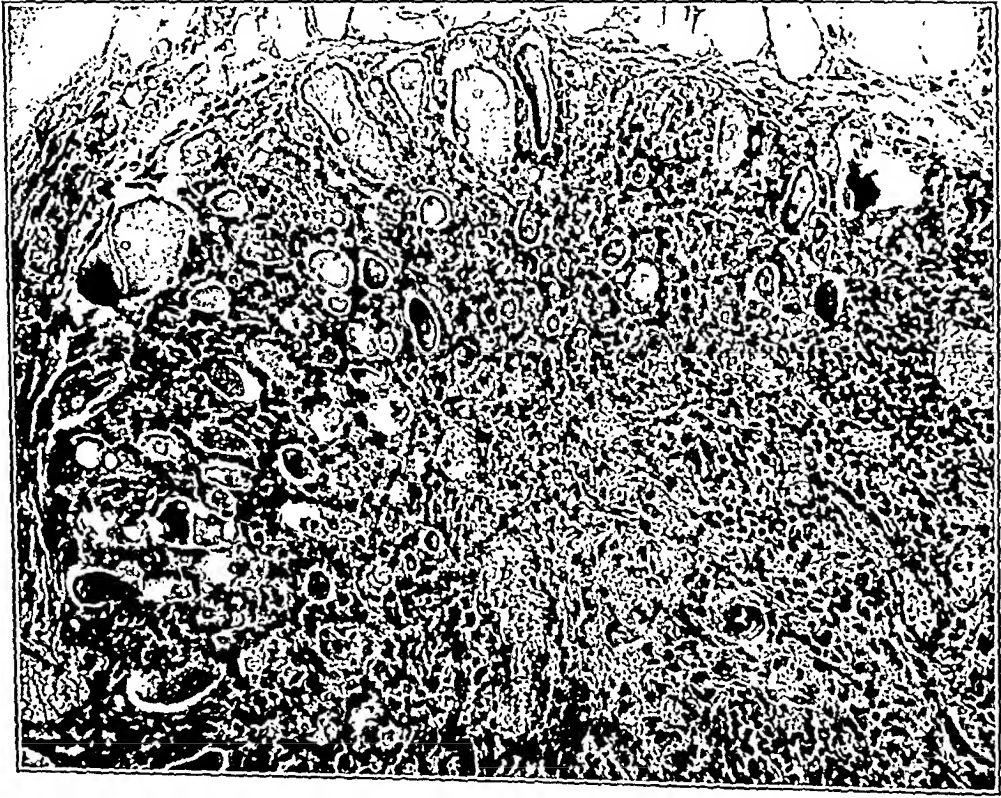
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dungzellen" were identical with the endothelial cells of the lymph sinuses, but at the same time thought that they might be fibroblasts. They also stated that reticulum was changed to collagen by a metaplasia under certain conditions.

In the same year and by similar methods, Russakoff ⁴ came to essentially the same conclusions.

Miller ⁵ in 1923 studied the reticulum content of tubercles in the lung and expressed the opinion that reticulum, while different from collagen, was apparently often changed into the latter.

This hypothesis was also upheld by Foot ⁶ in a similar study in 1925. Foot's opinion as to the formation of reticulum is that it does not exist as fibrils connected with cells, but is precipitated out in some manner in an intercellular secretion. He as well as several other pathologists apparently regard the reticulo-endothelial cell as the one which forms these fibrils because their diagnoses of the so-called reticulo-endotheliomas are based on the presence and arrangement of these fibrils in certain tumors.

METHODS

¶ In view of the differences of opinion in regard to the origin and nature of reticulum, it seemed to us worth while to undertake a comparative study of it by utilizing not only the silver stains but also all the other recognized staining methods for demonstrating intercellular substances.

We made use almost exclusively of tissues which were excised surgically in order to obtain them as fresh as possible. Thin sections of these tissues, not over 2 to 3 mm. thick, were cut and placed in Zenker's fluid within a few minutes after removal from the body and carried through in the usual way. Paraffin sections were employed and the following stains used on each specimen: Foot's modification of the Bielschowsky-Maresch silver impregnation method,⁷ Van Gieson's picro-acid fuchsin mixture,⁸ and Mallory's eosin or phloxin-methylene blue,^{8a} anilin blue collagen,^{8b} phosphotungstic acid and phosphomolybdic acid hematoxylin stains.^{8c} In addition a limited number of sections were stained using Verocay's technic ⁹ and the different methods specific for elastic fibrils. Frozen sections of a few tissues fixed in formaldehyde were stained by the original Bielschowsky-Maresch method ^{9a} and by Perdrau's ¹⁰ modification of it.

RETICULUM *

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INTRODUCTION

In recent years one particular tissue, the so-called reticulo-endothelial system, has been attracting increasing attention both from the physiologic (immunologic) and from the anatomic point of view. Although this tissue has been extensively studied, the resultant views of the different workers in regard to it are by no means in agreement. The two distinctive elements of this system are the endothelial cells and the framework or reticulum of intercellular fibrils which support them. In this paper we are concerned only with the origin and nature of the reticulum. The two undecided questions with regard to it are these: 1. Is reticulum the same as collagen or is it a chemically different substance which may be transformed into collagen? 2. Is reticulum produced by endothelial cells, by so-called reticular cells, or by fibroblasts?

HISTORICAL

Under the name of *Gitterfasern*, Kupffer¹ in 1876 described the reticulum occurring in the liver.

Mall² in 1896, as the result of digestive experiments and chemical analyses, concluded that there were three kinds of connective tissue fibrils, elastic, collagenous and reticular. He felt that each of these was a distinct variety. He found much reticulum in the capsule and trabeculae of the spleen, but none in the pulp. The exact reverse of these conditions was obtained by later workers who used silver stains.

In 1908 Rössle and Yoshida³ studied the reticulum of lymph nodes and other organs by means of the Bielschowsky-Maresch silver impregnation method and the Van Gieson stain, and decided that reticulum was closely related to collagen but not identical with it. They found that collagen and reticulum fibrils were often continuous with one another and they felt that the "*Gitterfasernbil-*

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ticeable that the fibrils when occurring singly or in delicate strands stained black, but where they were compacted into bundles, as around blood vessels, they stained only a reddish color.

In a series of leiomyomas of the uterus the same condition was found. Where the layer of intercellular fibrils between the muscle cells was thin and delicate it was stained intensely by the silver method and appeared black. Wherever the muscle cells had died off and disappeared so that the intercellular fibrils were compacted, they no longer stained black but were colored reddish like ordinary collagen. It, therefore, seems reasonable to conclude either that the reticulum was originally collagen or that it had turned into collagen when the muscle cells had disappeared.

Even in the rapidly growing tumors of this type, the leiomyosarcomas, there is the same formation of a reticulum composed of delicate collagen fibrils which must be supplied by the fibroblasts of the stroma. No other cell in the body seems to make such demands for an intercellular substance to surround and support it.

A study of the smooth muscle cells occurring in blood vessels, the gastro-intestinal tract and elsewhere revealed the same peculiarity of staining of the surrounding stroma by the silver method. Where the stroma was in a thin layer it stained black; where it was abundant following the disappearance of the muscle cells, and the formation of patches of sclerosis, it stained light red.

The application of all the other stains for intercellular substances showed that reticulum and collagen stain in exactly the same manner. Morphologically they differ only in the compactness of the fibrils.

The foregoing observations show therefore that smooth muscle cells under all conditions tend to be invested by a thin layer of collagen fibrils which stain black by the silver method. Because of this staining peculiarity and the arrangement around the cells as seen in cross-section, the collagen fibrils are called reticulum. When the reticulum fibrils are compacted owing to the disappearance of muscle cells they stain like collagen fibrils with which morphologically they are identical.

FIBROBLASTOMA

In rapidly growing fibrosarcomas, the collagen produced by the tumor cells is very slight in amount, and is stained black by the silver method. In cross-sections the bundles of cells are seen to be

In our work we chose tumors for examination because the amount of original tissue in them is obviously slight. Furthermore, tumors tend to grow as a single type of cell with only a supporting stroma of blood vessels and connective tissue. In describing our results we shall consider the various types of tumors studied and then the tissues of the reticulo-endothelial system.

With regard to the Bielschowsky silver method, it should be stated that it does not stain fibrin or neuroglia, fibroglia, myoglia or elastic fibrils. Moreover, the results obtained with this method at the edges of the sections where the fixative acted first are not reliable.

Phosphomolybdic acid hematoxylin, like the Verocay method, brings out quite distinctly all the reticulum and collagen in the tissues and has the great advantage of neither causing shrinkage nor freeing paraffin sections from the slide. The best formula for staining collagen seems to be the following:

Water	100 cc.
Phosphomolybdic acid	2 gm.
Hematoxylin	1 gm.

The hematoxylin dissolves readily in the acid. The solution can be ripened for immediate use by the addition of five cc. of a one per cent solution of permanganate of potassium.

Stain paraffin sections of Zenker-fixed tissue for twenty-four hours in the cold, or, if a more intense stain is desired, in the paraffin oven at about 54° C. for two to three hours. Wash in water, dehydrate in ninety-five per cent alcohol followed by absolute, clear in xylol and mount in xylol balsam.

LEIOMYOBLASTOMA

Silver stains of a leiomyoma arising from the wall of a vein in the groin showed that each muscle cell, when examined in cross-section, was separated from its neighbors by a thin layer of delicate black-staining fibrils. In other words the muscle cells were completely invested in what is called reticulum. On staining sections, already impregnated with silver, by the anilin blue collagen method it could be seen that the myoglia fibrils were stained red by the acid fuchsin and lay inside of the black reticulum. Where the bundles of muscle cells were running lengthwise, the reticulum was disclosed as delicate wavy fibrils differing morphologically in no respect from the fibrils which form the bundles of ordinary collagen. It was no-

because they course in different directions over the surface of the anastomosing fibroblasts. In the scirrhus type of lymphoblastoma, the reticulum is smaller meshed and often contains many eosinophiles as well as other leucocytes in addition to the tumor cells. In a later stage, as the cells disappear owing to degeneration or emigration, the strands of the reticulum are approximated and stretched more or less definitely in one direction or another. Under these conditions they appear and stain as collagen.

THE RETICULO-ENDOTHELIAL SYSTEM

The information derived from the study of the staining reactions of the intercellular fibrils of certain tumors was applied to the organs of the reticulo-endothelial system.

The stroma of lymph nodes aside from the blood vessels consists of a network of anastomosing fibroblasts which form a syncytium. The collagen produced by the fibroblasts is arranged in a reticulum composed of strands of delicate fibrils which course over the surface of the cells in different directions. Endothelial cells are applied in places to the surface of the reticulum which stains black by the silver method. The lymphocytes are contained in the meshes of the reticulum.

When the axillary lymph nodes are invaded by a scirrhus carcinoma, the stroma cells react as fibroblasts and produce an abundance of collagen.

The capsule and trabeculae of the spleen contain much collagen, many fibroglia and elastic fibrils but no reticulum. The lymph nodules are like those in lymph nodes, and the arteries and veins are similar to the blood vessels elsewhere in the body. The peculiar and characteristic feature of the spleen is the presence of the blood sinuses. They are lined with endothelial cells resting on a stroma of delicate strands of collagen which, on account of its occurrence in this form, stains intensely by the silver method. The fibroblasts which produce the reticulum are characterized by the presence of fibroglia fibrils.

In the organization of infarcts of the spleen, the fibroblasts of the stroma of the pulp produce scar tissue containing much collagen.

surrounded by a delicate reticulum, but in longitudinal sections, the reticulum is resolved into delicate wavy fibrils. Stated differently, collagen is stained black by the silver impregnation method when it occurs in single fibrils, in very thin layers or delicate strands.

In more slowly growing fibrosarcomas and in fibromas, the collagen does not stain black, but has a reddish color. In a slowly growing fibromyxosarcoma, on the other hand, the collagen stains intensely black wherever it is separated into individual fibrils or fine strands by the homogeneous intercellular substance.

The tumors of this group, producing their own intercellular fibrils, demonstrate clearly that collagen stains specifically by the silver impregnation method only when it occurs in single separated fibrils or in fine strands or thin layers.

CARCINOMA

We chose scirrhus cancers of the breast in order to study the stroma. The fibroblasts are always well developed with numerous fibroglia and collagen fibrils. In addition the elastic fibrils are often abundant in places. The silver preparations showed great numbers of black-staining fibrils around and often between the groups of tumor cells, in the masses of elastic tissue and in the stroma wherever there was edema or infiltration with leucocytes; but no black-staining fibrils were seen where the collagen occurred in coarse bundles or solid masses. Furthermore it was evident everywhere that the black-staining fibrils were always in direct connection with the lightly stained collagen.

With all the other staining methods for intercellular substances, collagen and reticulum reacted and stained in the same way.

LYMPHOBLASTOMA

The stroma of a rapidly growing lymphoblastoma consists of a delicate network of intercellular fibrils in the meshes of which lie the tumor cells. The finer threads of this network are stained black by the silver impregnation method, while the coarser are colored black to reddish according to their compactness. The network consists of collagen fibrils arranged in fine or coarse bundles. They are closely applied to the fibroblasts which form a syncytium. The collagen fibrils do not branch, but the bundles of them often seem to branch,

The different views in regard to reticulum will be taken up seriatim.

1. *Is reticulum a precollagenous substance?*

In favor of this view is the early appearance of the reticulum in tubercles and in granulation tissue. In these pathologic conditions the reticulum is in direct continuity with the surrounding collagen into which it changes at a later stage of the process. On the other hand, reticulum persists for a lifetime around smooth muscle cells and yet apparently changes at once to collagen if the cells it surrounds atrophy and disappear as, for example, in leiomyomas. The only change which has happened to the reticulum is a physical one: its fibrils have been brought into close contact with one another; in other words, they have been compacted.

2. *Is reticulum produced by reticular cells?*

This conception would necessitate the recognition of a new type of cell which produces a fibrillar intercellular substance composed of delicate fibrils exactly similar to those produced by the fibroblast, which change to collagen when they are closely packed together.

3. *Is reticulum produced by endothelial cells?*

This view has attained great vogue recently and is strongly advocated by Aschoff¹¹ in particular. It might be conceivable if one were to limit one's studies to the liver, spleen and lymph nodes; but not if one were to consider all the other organs and tissues of the body. It would mean that the intercellular fibrillar substance in a rapidly growing fibrosarcoma, which is stained black by silver, is produced by the endothelial cells of the blood vessels and not by the fibroblasts of the tumor. In the more slowly growing tumors of this type, however, it is produced entirely by the tumor cells because only collagen is present. It would also indicate that endothelial cells in the stroma of a scirrhus cancer of the breast produced the reticulum around and between the epithelial cells of the tumor at a considerable distance from the blood vessels.

4. *Is reticulum produced by fibroblasts?*

There are several points in support of this view. In a rapidly growing fibrosarcoma, the fibrillar intercellular substance stains like reticulum. In the more slowly growing tumors it stains like collagen and yet the age of the intercellular substance evidently has nothing to do with it. If the fibrils of a slowly growing fibrosarcoma are

LIVER

The connective tissue of the liver extends from around the portal and hepatic vessels through the lobule in the form of delicate strands of collagen, which stain black by the silver method. These strands which surround the columns of liver cells lie between them and the endothelial cells lining the sinusoids. They are produced by fibroblasts. In certain types of cirrhosis of the liver, the reticulum of the stroma stains like collagen when it is compacted into patches of sclerosis as the result of degeneration and disappearance of liver cells. When the stroma of the liver is increased in amount as the result of infectious processes (tuberculosis, infectious cirrhosis), collagen is produced in abundance.

In blood vessels, reticulum is present beneath the lining endothelium and between the muscle cells and extends out around the capillaries forming a delicate sheath about them. Reticulum is not formed by the endothelial cells but by the fibroblasts of the stroma for the support of the vessels, in exactly the same manner that it is supplied for the support of smooth muscle cells wherever they occur in the body.

DISCUSSION

The recognition of reticulum depends on two characteristics, structure and staining reaction. The name was originally applied to the fibrillar intercellular substance which forms a network composed of fine strands for the support of cells in various organs and tissues. Later it was discovered that the silver impregnation method, variously modified, stained reticulum black while collagen took on a light brownish or reddish tint according to the method employed. On this account it was generally assumed that the stain was specific, and that reticulum and collagen were chemically different substances. Therefore, the Bielschowsky silver stain in recent years has become the standard means for the recognition of reticulum and has proved a great stimulus to the study of its distribution. It should be noted, however, that reticulum and collagen react alike to all other stains for the demonstration of intercellular substances.

As stated in the introduction to this paper, there are different views in regard to the origin and nature of reticulum. The subject has been confusing, because of the evident close relation between reticulum and collagen, not only chemically but also morphologically. Both are composed of delicate fibrils which are often wavy.

black by the silver. When the muscle cells die, the black-staining fibrils around them disappear, but the collagen is increased in amount. The obvious inference is that separated collagen fibrils are stained by silver, but compacted fibrils are not. Myoglia fibrils are not stained by silver. This point can be demonstrated by using the acid fuchsin-anilin blue method on a section already treated with silver.

In fibrosarcomas the collagen is stained black by silver, if it is slight in amount. It is not stained when present in large amounts.

In cancers many loose fibrils surrounding and running between the epithelial cells stain black; the same is true of single fibrils or very small bundles of them embedded in elastic tissue. Stains for fibroglia fibrils following silver stains show that they are not colored by the silver.

The capsule and trabeculae of the spleen contain much collagen, many fibroglia and elastic fibrils but no reticulum. The stroma of the lymph nodules and pulp stains black. Stains for fibroglia fibrils show them to be present running along the surface of the reticular stroma. Hence the cells forming this network are fibroblasts.

It is generally agreed that physically reticulum and collagen are intimately joined together, being always continuous one with the other.

Many believe that reticulum changes to collagen. All reticulum stains by the methods used for collagen, namely, Van Gieson's, Verocay's, anilin blue and phosphomolybdic hematoxylin.

The silver stains are specific for collagen but only under certain physical conditions. Collagen must be separated into individual fibrils, or into very small strands and thin layers by cells, elastic fibrils or fluid.

Reticulum as a chemically distinct intercellular substance does not exist; it is collagen in separated form, rendered prominent by the silver stain.

All collagen is produced by fibroblasts.

There are no reticular cells other than fibroblasts.

Endothelial cells do not produce an intercellular substance.

separated by fluid or mucin, the collagen fibrils in these parts stain black with silver and must be regarded as reticulum.

In the stroma of scirrhus cancers the fibroblasts produce abundant fibrillar intercellular substance which stains like reticulum or collagen according to whether the fibrils are separated or compacted.

The most important point, however, is this: In its active condition, the fibroblast is characterized by the presence of fibroglia fibrils which run along its surface and which can be demonstrated by means of phosphotungstic acid hematoxylin or the acid fuchsin-anilin blue collagen method. Study of the so-called reticular cells of the spleen, lymph nodes and other organs show that they possess fibroglia fibrils and that they, therefore, are fibroblasts. For this purpose absolutely fresh tissue, just removed from the living body, cut into very thin sections 1 to 2 mm. thick and fixed in Zenker's fluid, must be used.

From this presentation of the results of a comparative study of the fibrillar intercellular substances present in various tumors and organs, it appears to us that reticulum is produced by fibroblasts and is merely collagen occurring as separated fibrils or as delicate strands. Only under this physical condition do the fibrils stain intensely by the silver impregnation method. When, as in leiomyomas, the fibrils of the reticulum are brought into close apposition through degeneration and disappearance of intervening cells, they no longer stain like reticulum but like collagen.

The Bielschowsky silver impregnation stain furnishes an excellent method for the demonstration of the finest fibrils of collagen, but the pictures it presents should be viewed with discretion to avoid misinterpretation. The results obtained by it must be controlled by other, more reliable methods which stain all the collagen present, even if not so intensely.

SUMMARY AND CONCLUSIONS

All recent work on reticulum has been based on the use of silver stains, chiefly Bielschowsky's and modifications of it. Our results depend on the use of tissue fixed immediately after removal from the living body, and on a comparative study of the various stains for intercellular substances.

Smooth muscle cells in leiomyomas, in the wall of blood vessels and elsewhere are surrounded by delicate fibrils which are stained

DESCRIPTION OF PLATE

All photomicrographs were made from sections of Zenker-fixed tissue, except Figs. 2 and 3; and stained by Foot's modification of Bielschowsky's silver method, except Figs. 1, 5, 16, 18 and 23.

PLATE 132

- FIG. 1. Leiomyoma originating from the wall of a vein in the inguinal region. Stained with phosphotungstic acid hematoxylin to show the myoglia fibrils. $\times 500$.
- FIG. 2. Cross-section of bundle of smooth muscle cells from the same tumor to demonstrate reticulum surrounding them. Formaldehyde fixation, Foot's silver method. $\times 1000$.

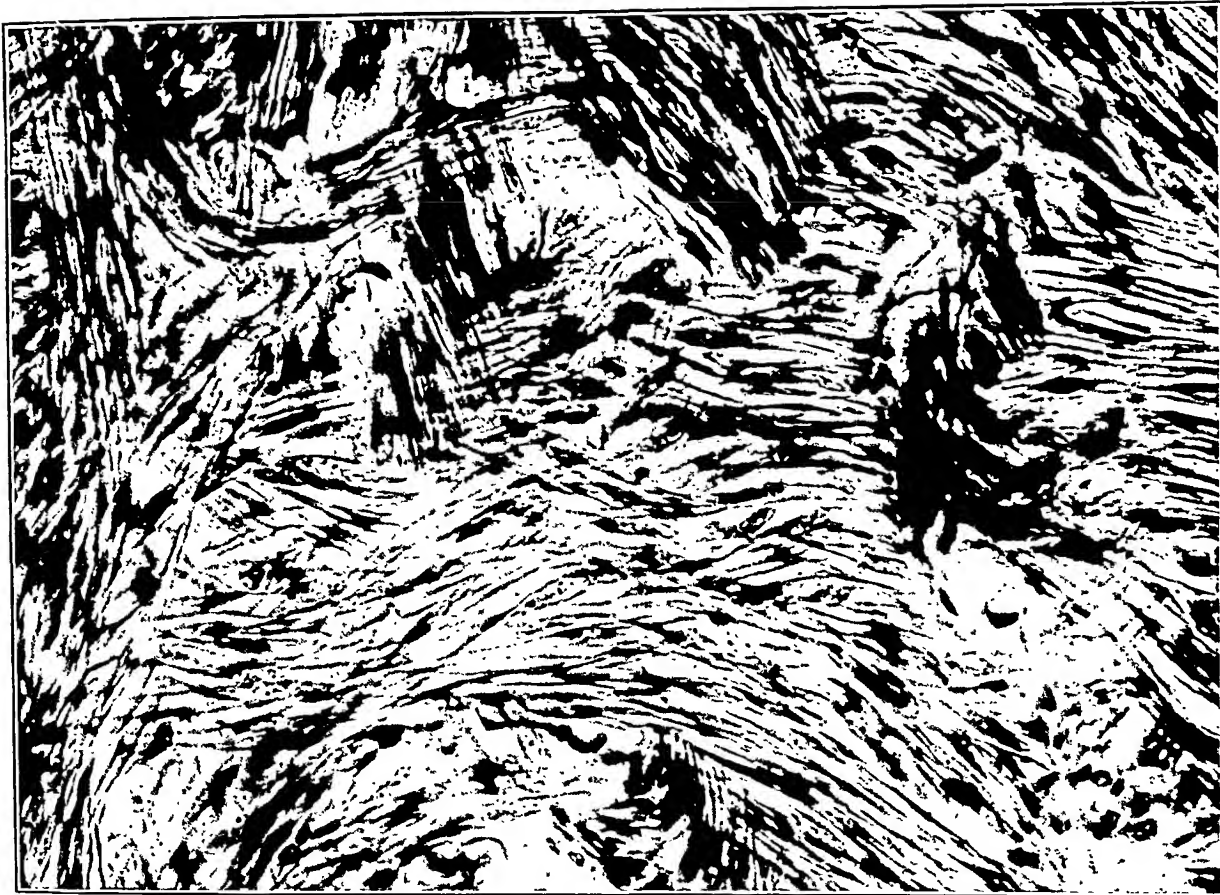
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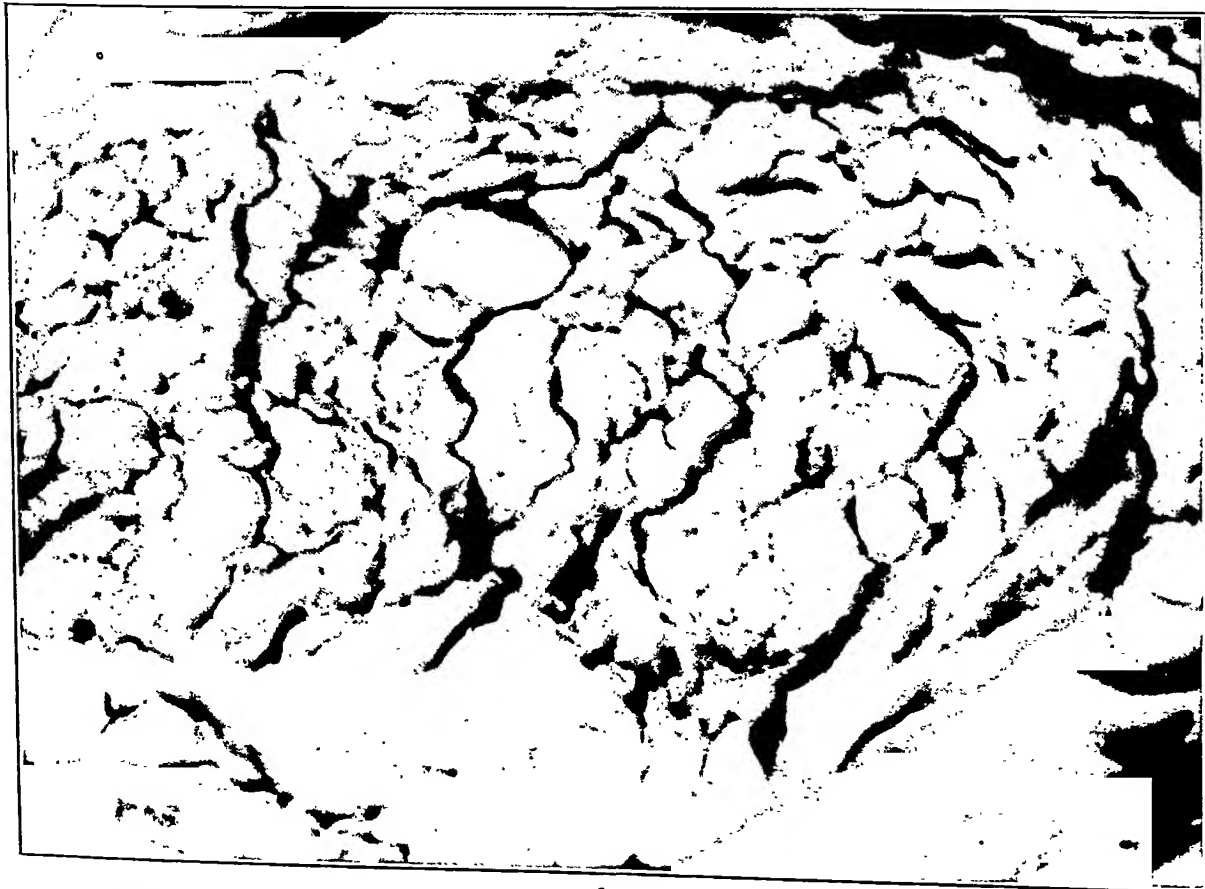
PLATE 133

FIG. 3. Longitudinal section of same leiomyoma (Figs. 1 and 2) to show reticulum resolved into wavy collagen fibrils occurring in single and in fine strands. Fixative and staining same as Fig. 2. $\times 1000$.

FIG. 4. Leiomyoma of uterus. Reticulum around muscle fibrils. Where the cells have degenerated and disappeared the reticulum fibrils have been compacted into collagen and stain very slightly. $\times 1000$.



1



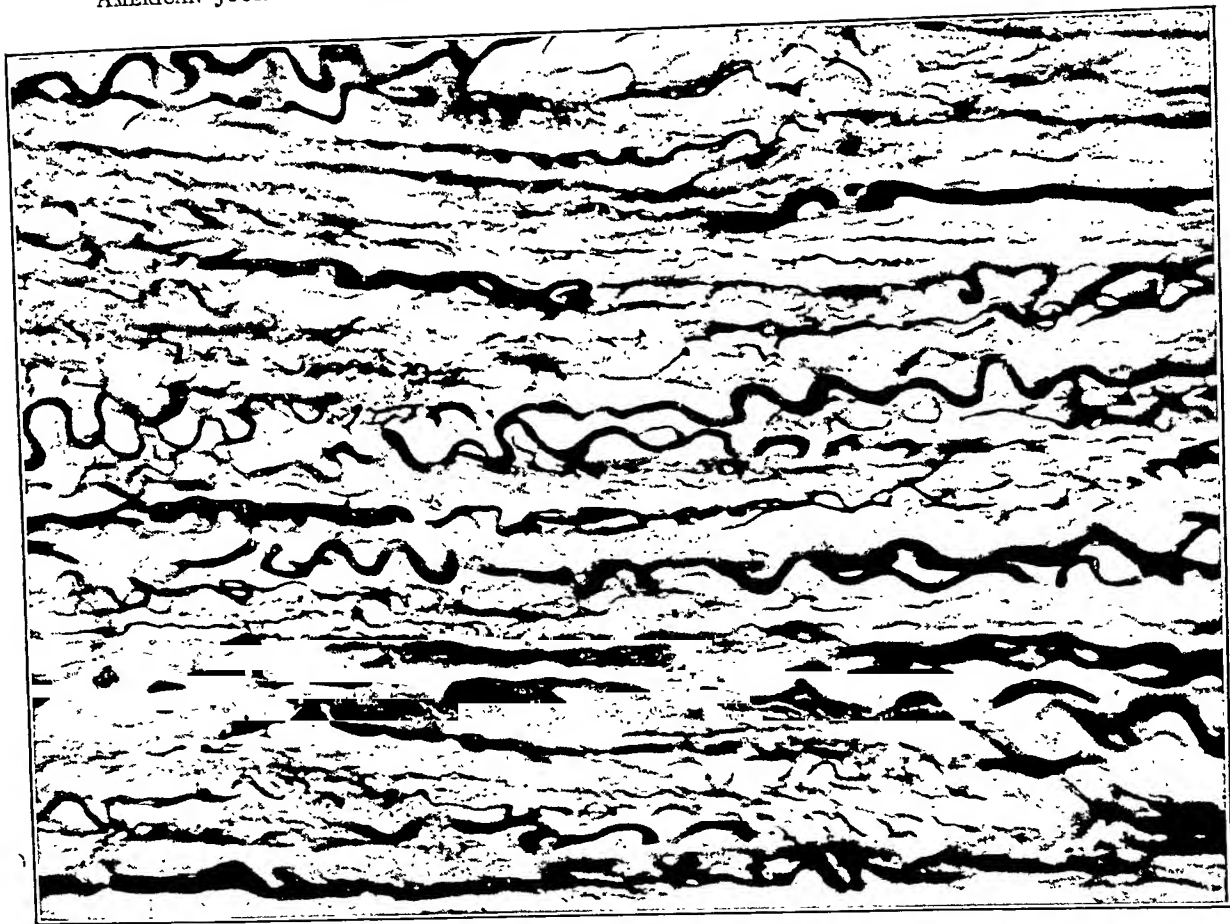
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Mallory and Parker

Reticulum

PLATE 134

- FIG. 5. From a metastasis in a cervical vertebra of a leiomyosarcoma of the uterus. Stained with phosphotungstic acid hematoxylin to show the myoglia fibrils. One diaster present. $\times 1000$.
- FIG. 6. From a metastasis of the same leiomyosarcoma to the orbit to show the reticulum between the cells. $\times 1000$.



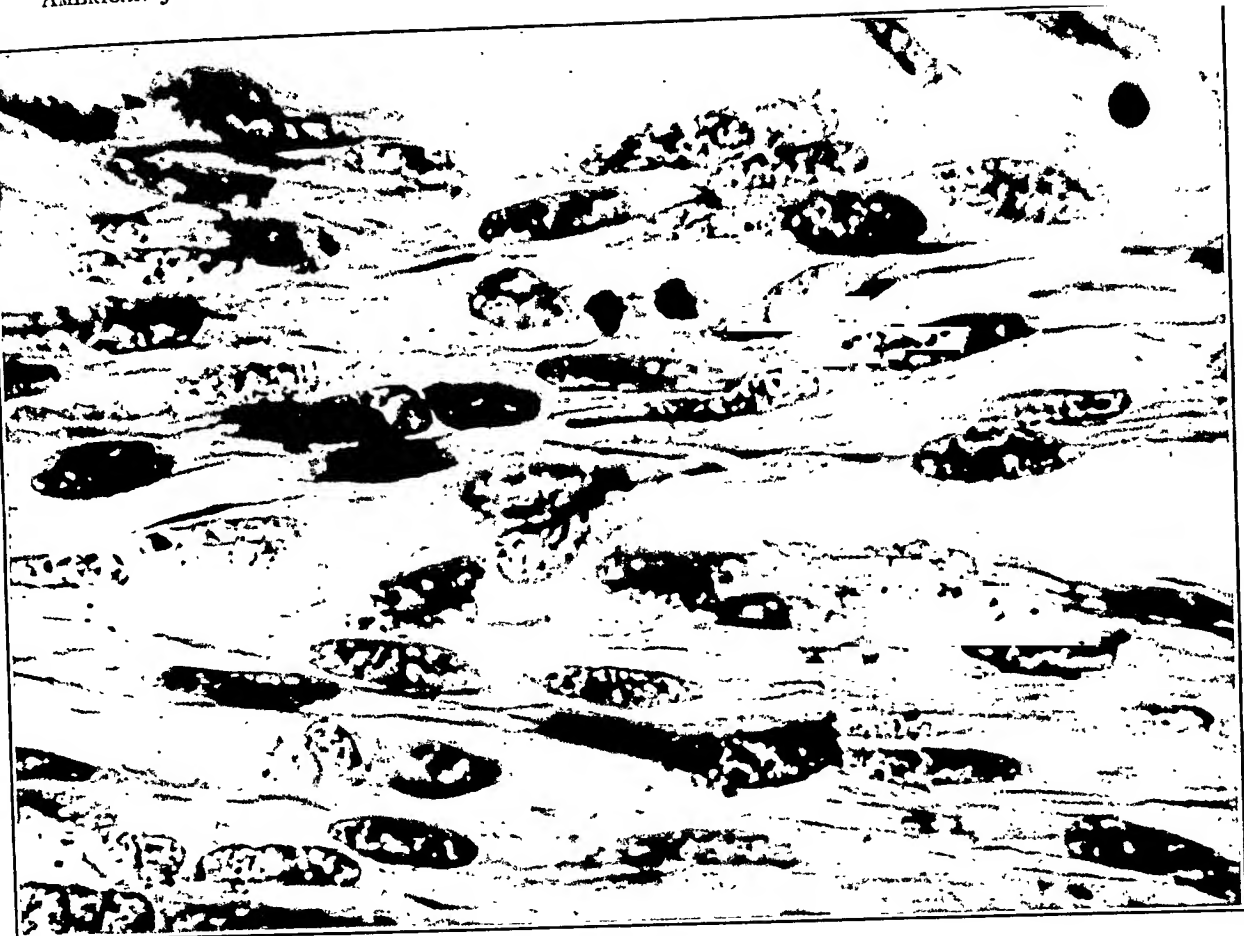
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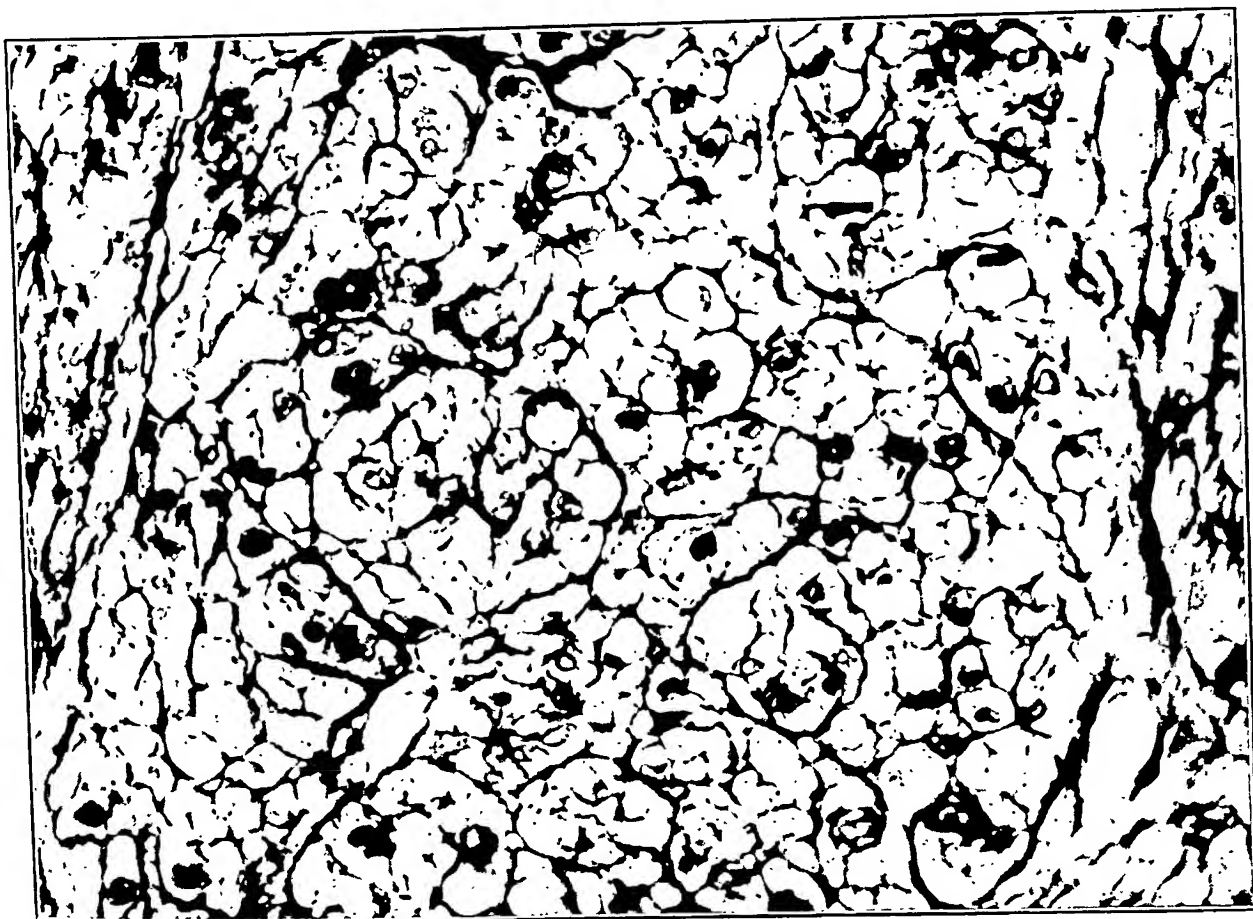
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PLATE 135

- FIG. 7. Longitudinal section of the metastasis (Fig. 5). The reticulum is seen here resolved into collagen fibrils. $\times 1000$.
- FIG. 8. A rapidly growing rhabdomyosarcoma projecting into a lymphatic. The fibroblasts of the stroma have furnished a reticulum surrounding single tumor cells or small groups of them. $\times 250$.



5

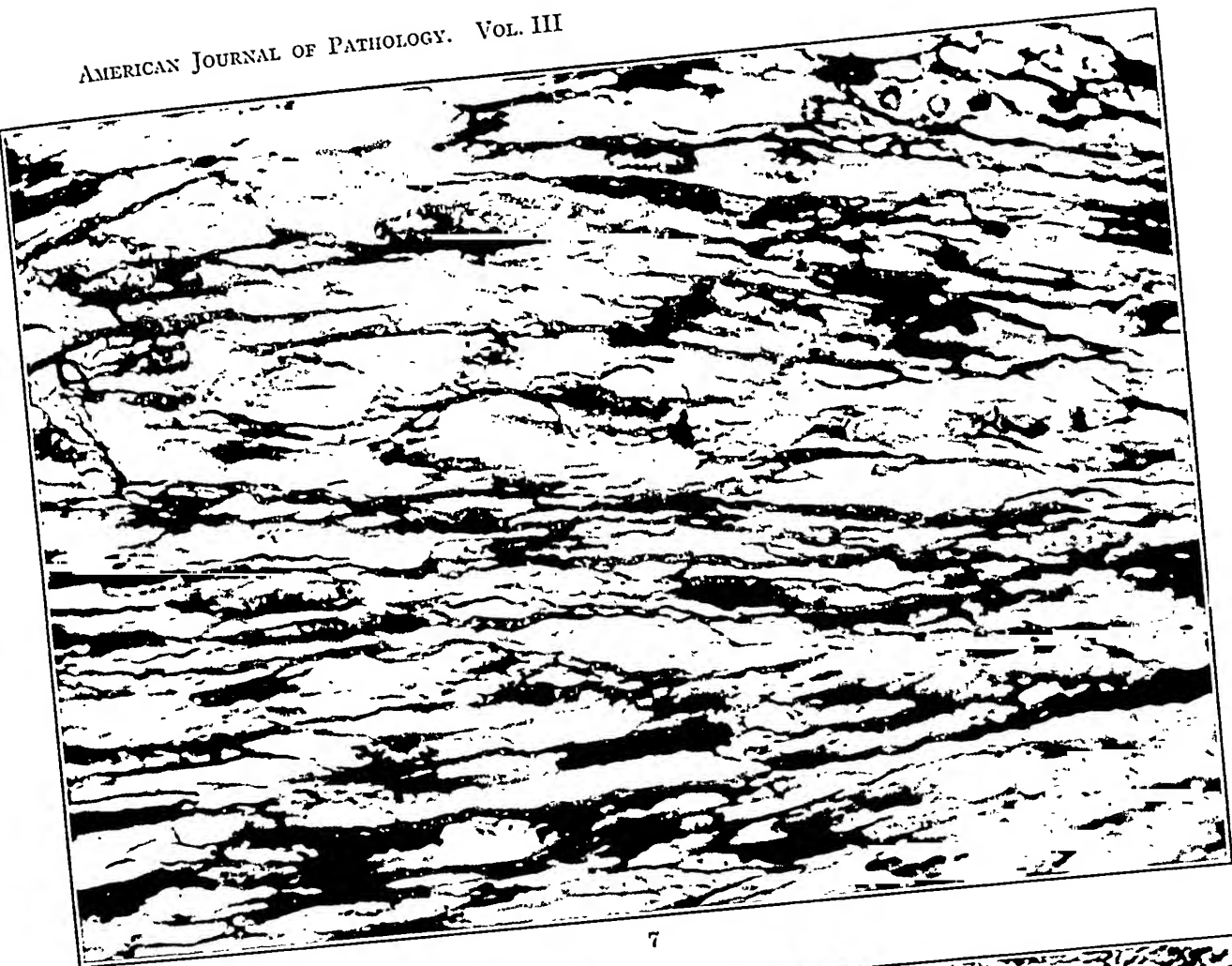


6

PLATE 136

FIG. 9. A rapidly growing fibrosarcoma of the kidney. The fibrillar intercellular substance is stained black by the silver method. One mitosis in the center of the field. $\times 500$.

FIG. 10. Fibrosarcoma of the pectoral muscle in a child. The fibrillar intercellular substance is stained black by the silver method. $\times 500$.



7



8

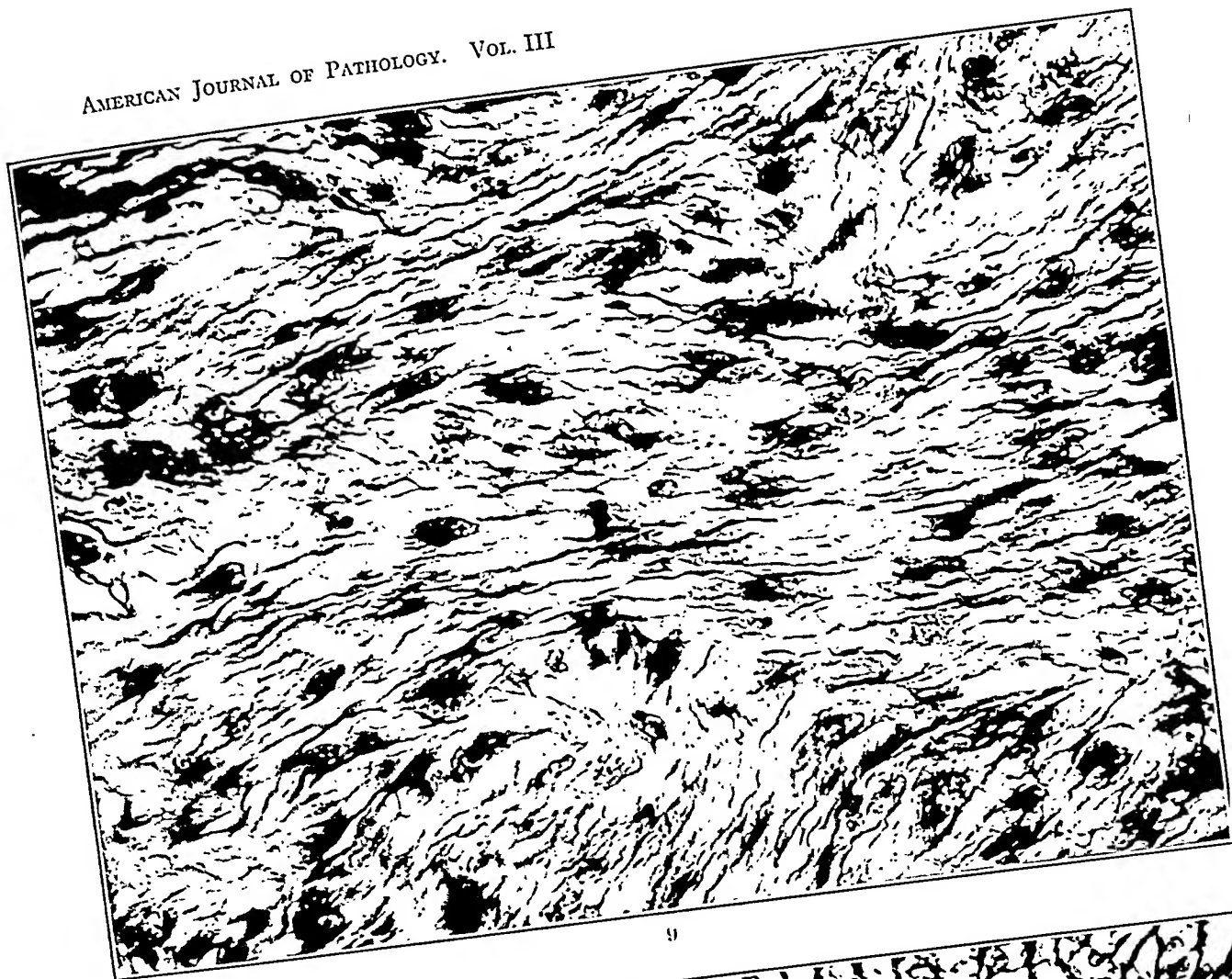
Reticulum

PLATE 137

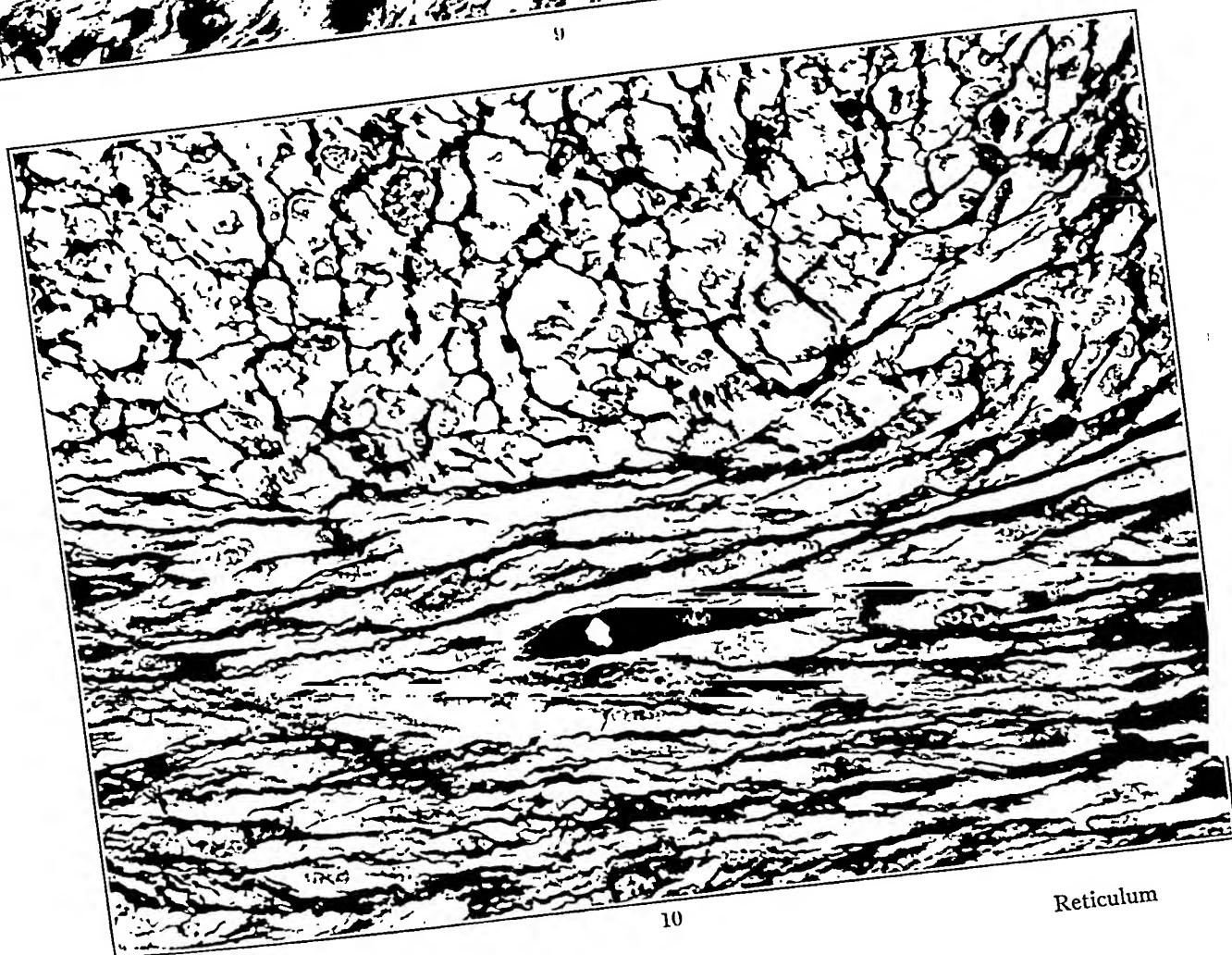
FIG. 11. Same tumor (Fig. 10) showing mitoses and one tumor giant cell.

× 500.

FIG. 12. A fibrosarcoma in which the fibrils run in all directions. All the finer ones stain black by the silver method, the coarser ones less intensely. The wavy fibrils in the center are in the wall of a dilated capillary. × 1000.



9



10

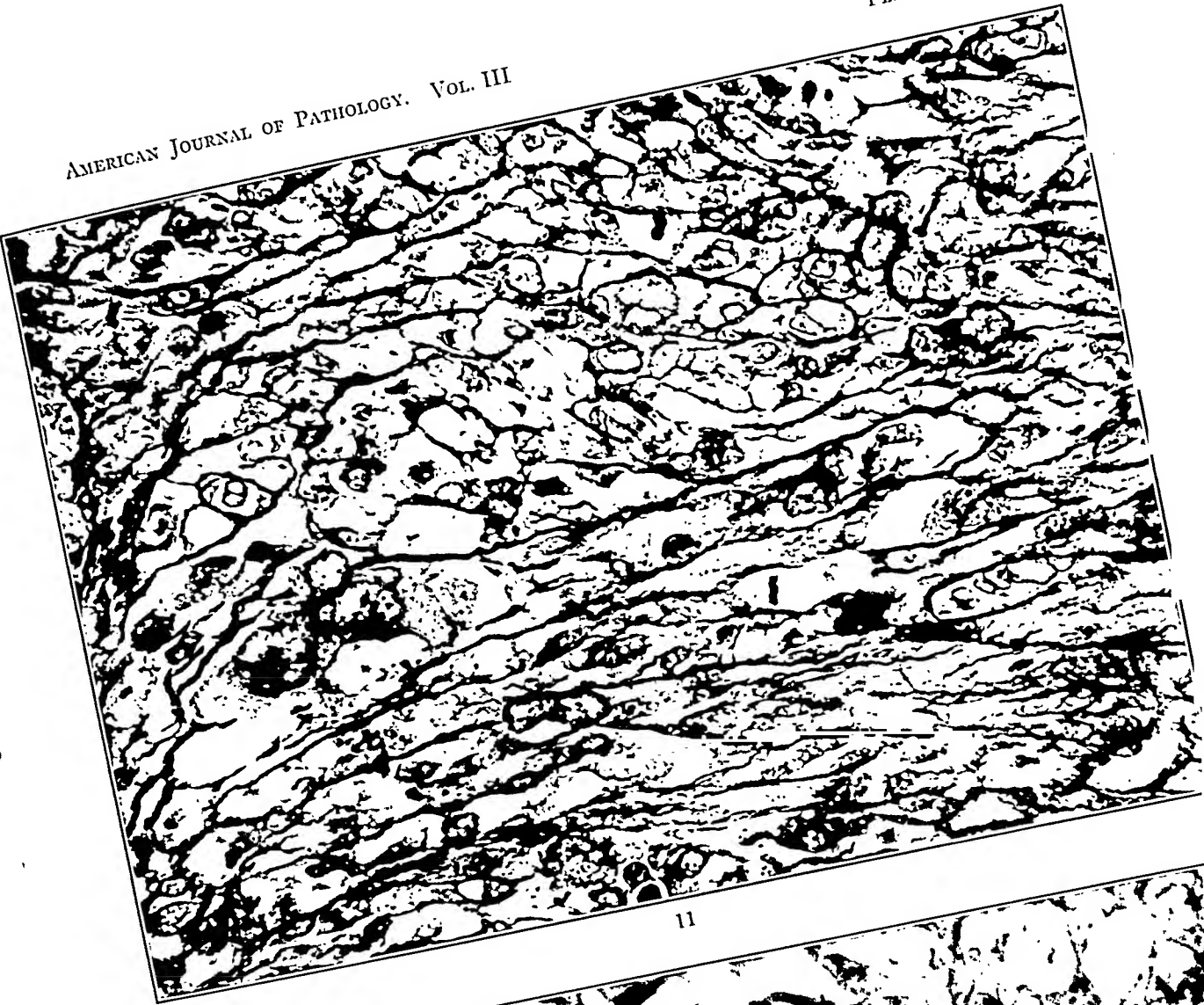
Reticulum

Mallory and Parker

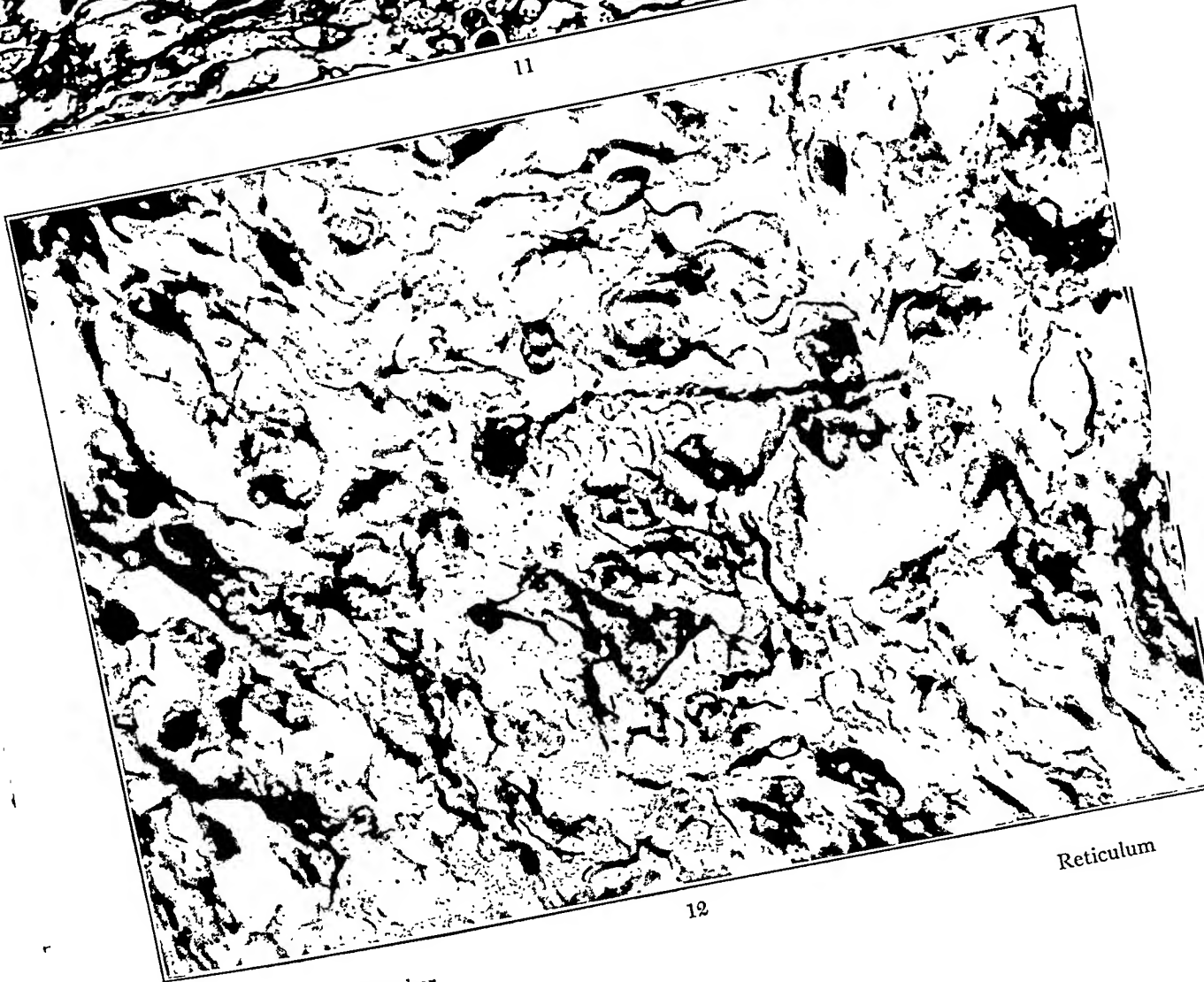
PLATE 138

FIG. 13. An edematous fibrosarcoma of the uterus. The separated fibrils all stain deep black by the silver method. $\times 500$.

FIG. 14. Fibromyxosarcoma. The fibrils in the myxomatous portion stain black. $\times 500$.



11



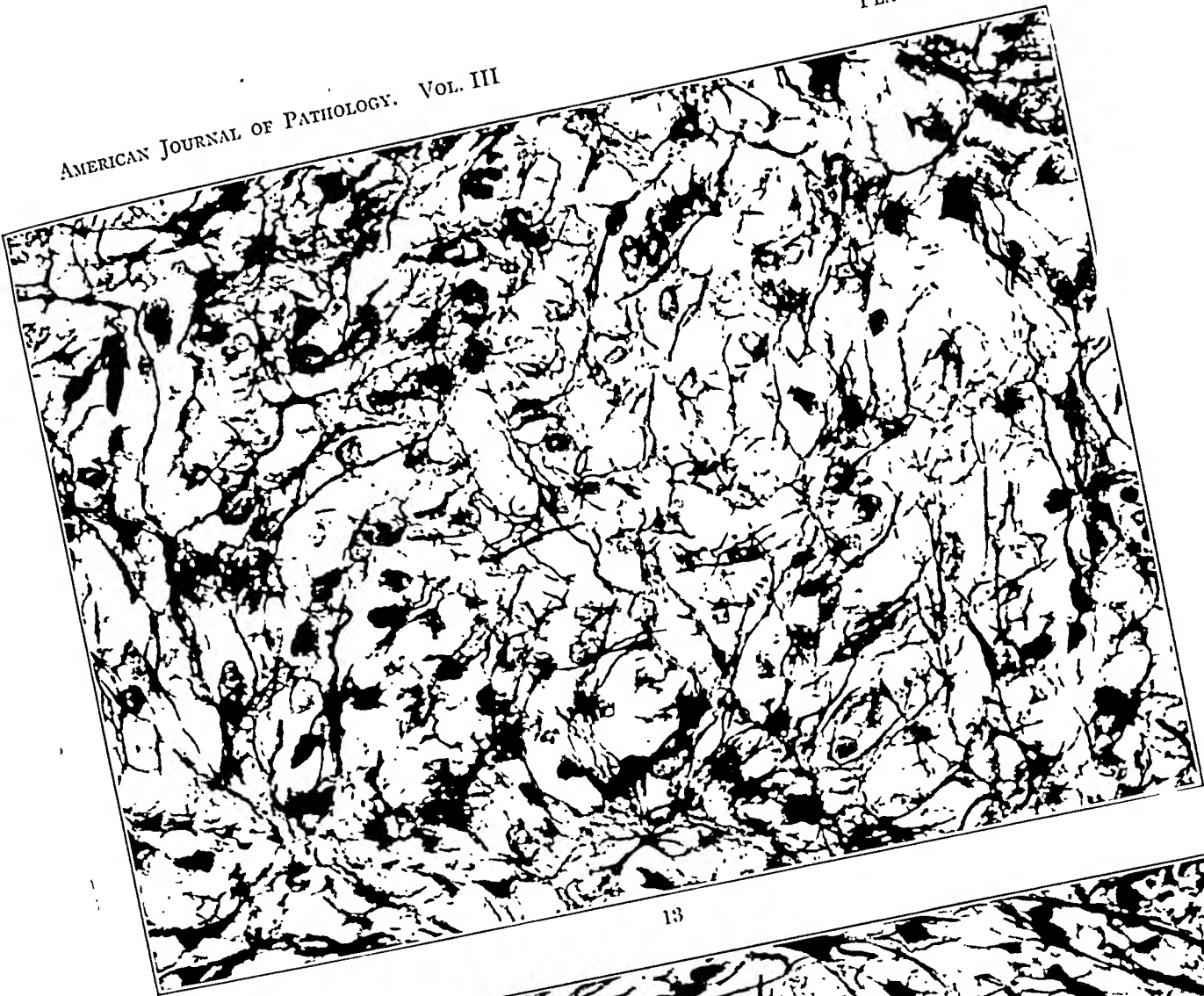
Reticulum

12

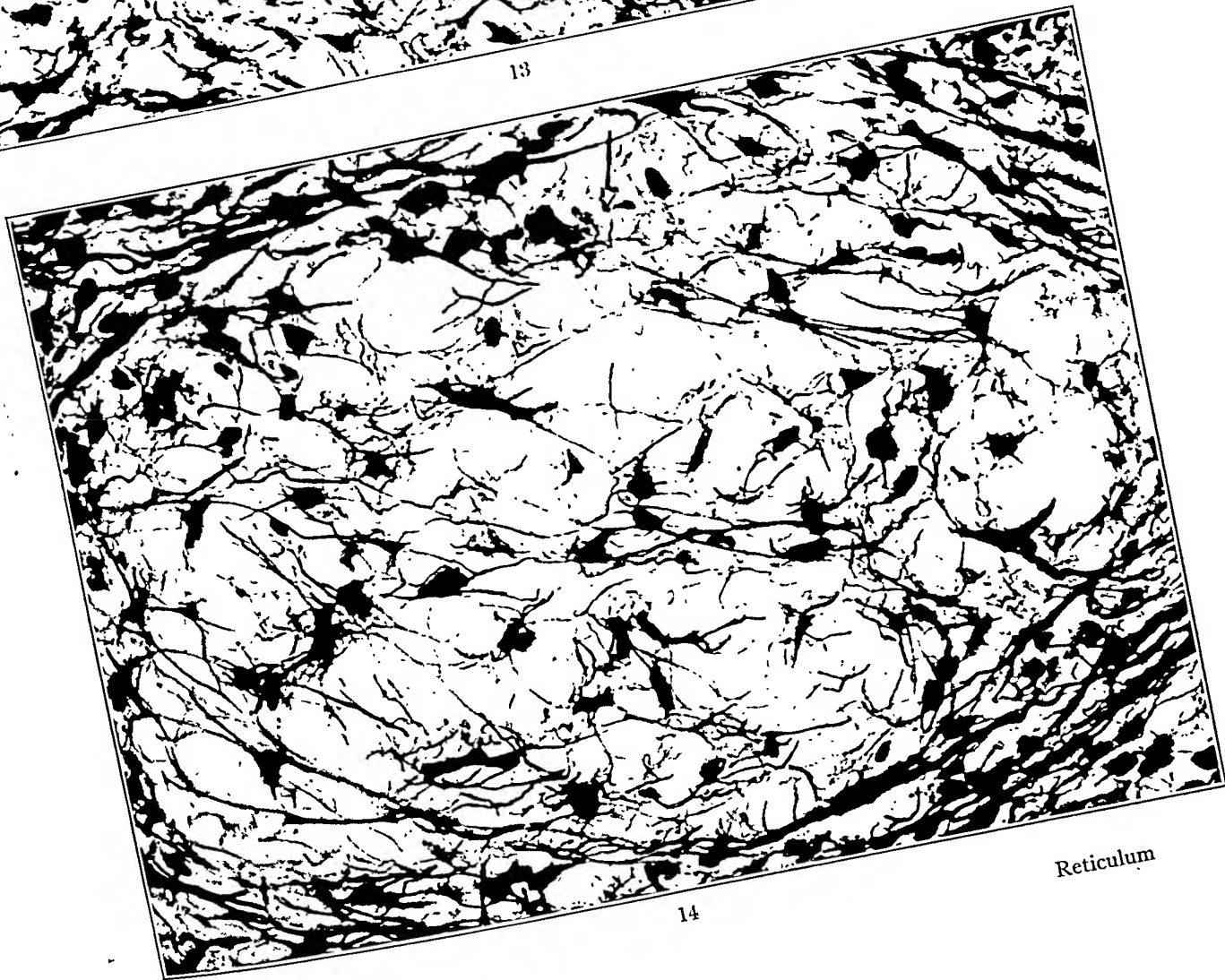
PLATE 139

FIG. 15. Lymphoblastoma. One mitosis present. The reticulum furnished by the fibroblasts of the stroma is stained black. $\times 500$.

FIG. 16. A phosphotungstic acid hematoxylin stain of the same tumor to demonstrate the fibroglia fibrils of the fibroblasts. $\times 1000$.



13

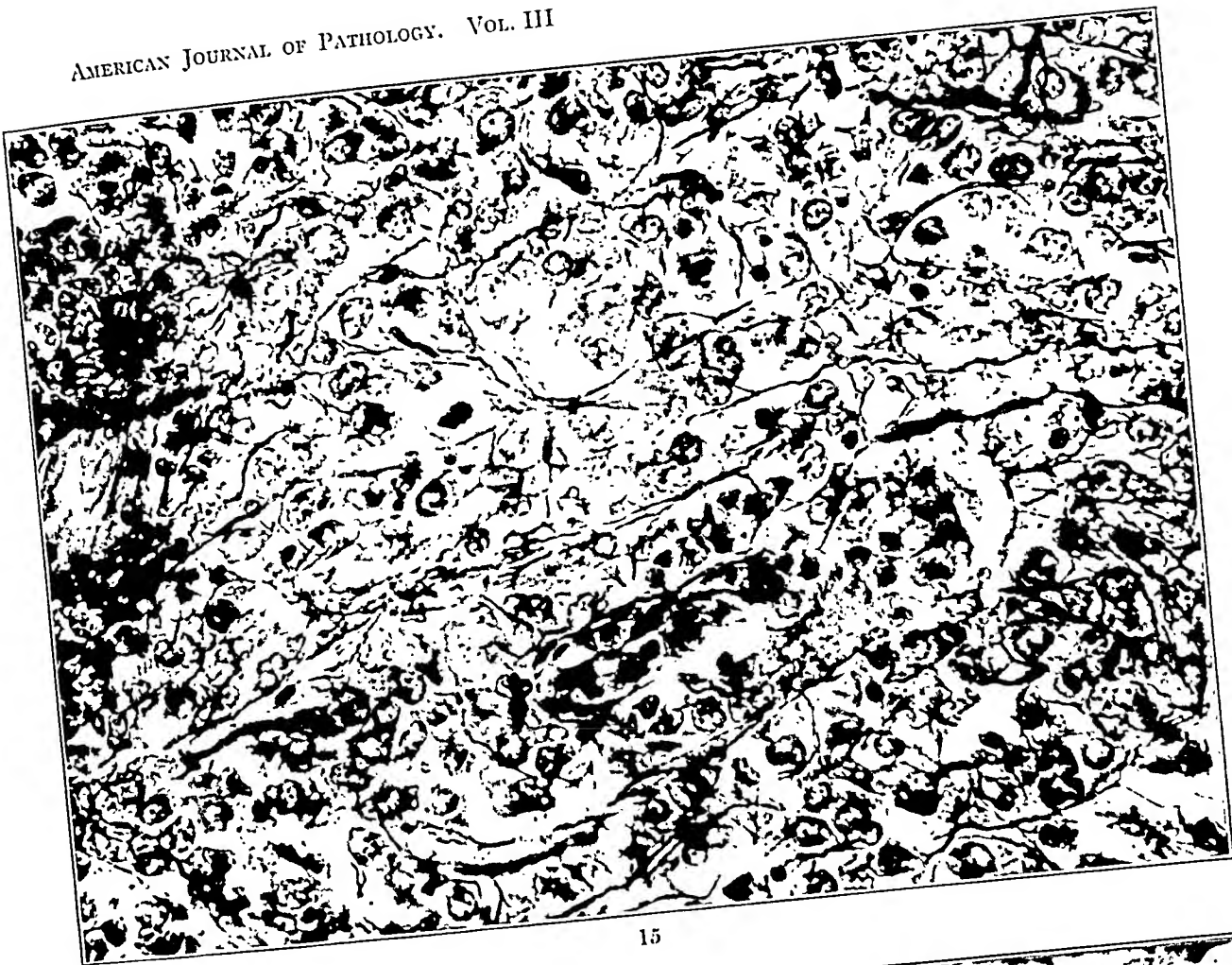


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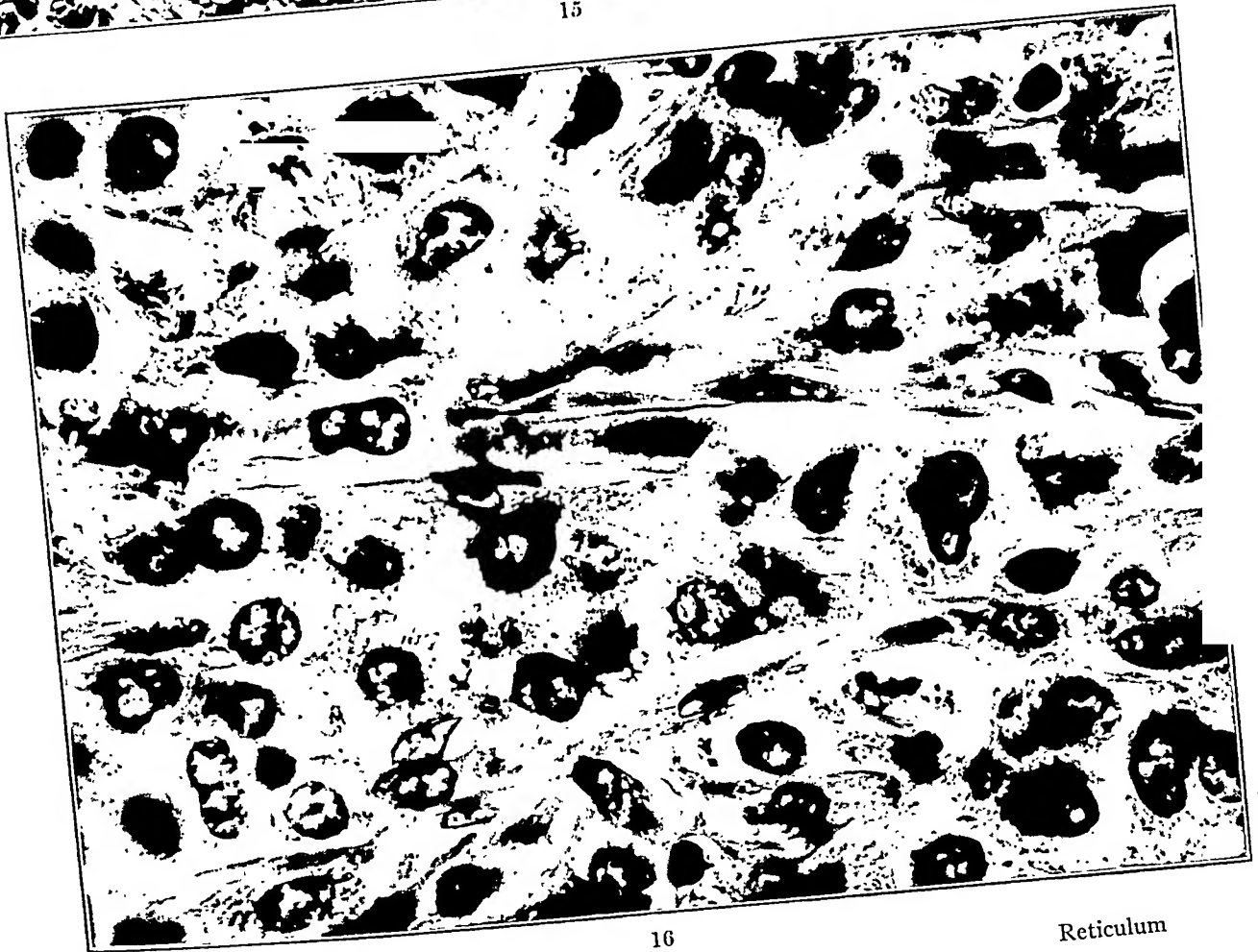
Reticulum

PLATE 140

- FIG. 17. A silver stain of the reticulum in a lymph nodule of the spleen. $\times 250$.
FIG. 18. Another lymph nodule in the same spleen stained by phosphomolybdic acid hematoxylin. $\times 250$.
FIG. 19. Cancer of the breast. The collagen fibrils running near and between the tumor cells are stained intensely black. $\times 500$.



15



16

Reticulum

Mallory and Parker

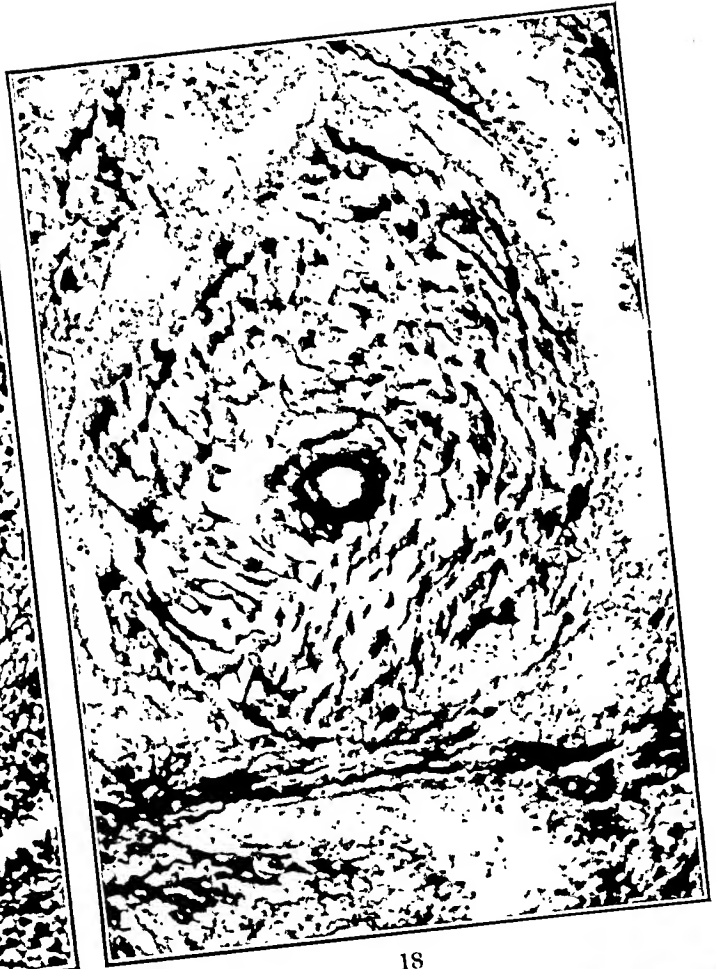
PLATE 141

FIG. 20. A similar condition is shown in another illustration from the same tumor (Fig. 19). $\times 1000$.

FIG. 21. In the same cancer the collagen fibrils, separated by elastic fibrils, stain intensely. $\times 500$.



17



18



19

Reticulum

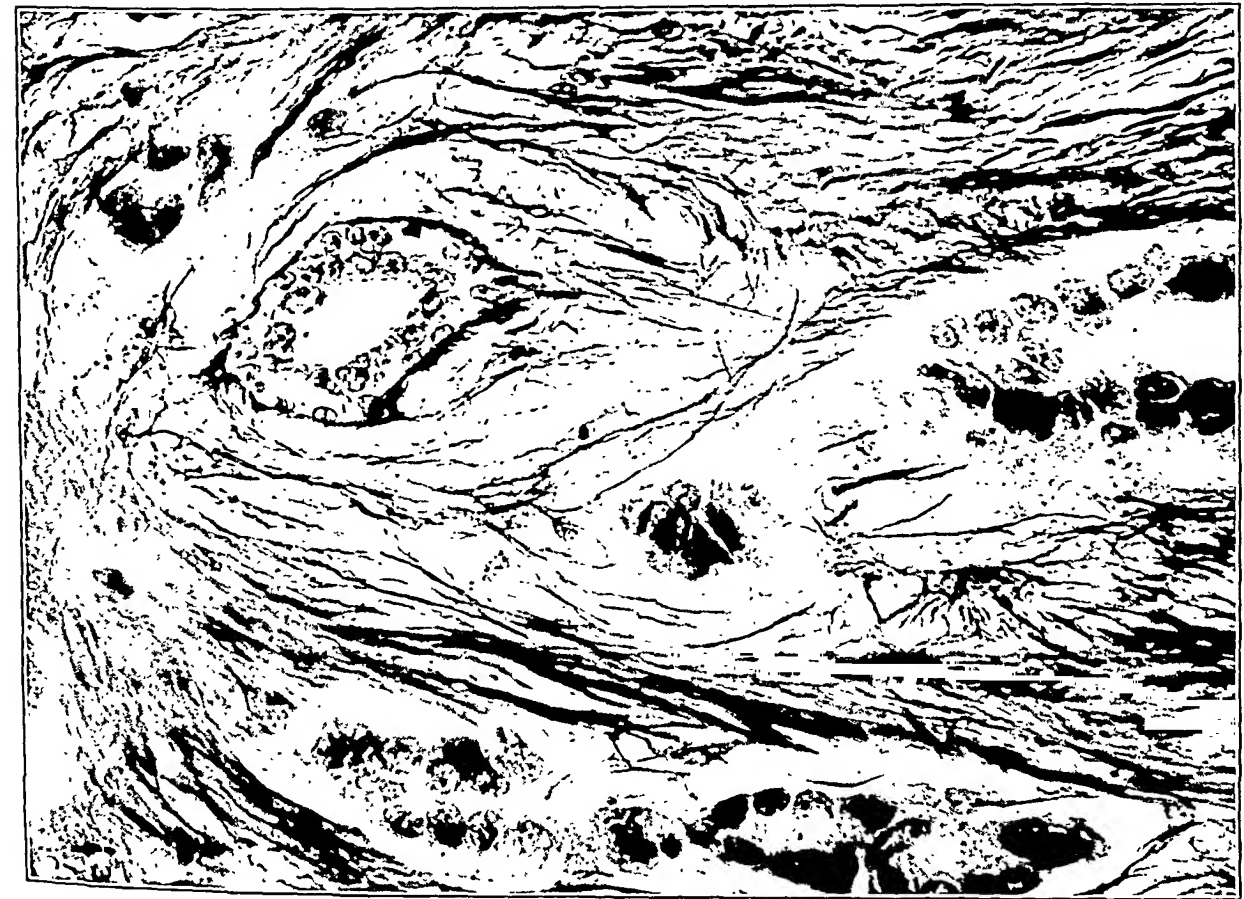
PLATE 142

FIG. 22. The same holds true of fibrils separated by an infiltration of lymphocytes. $\times 1000$.

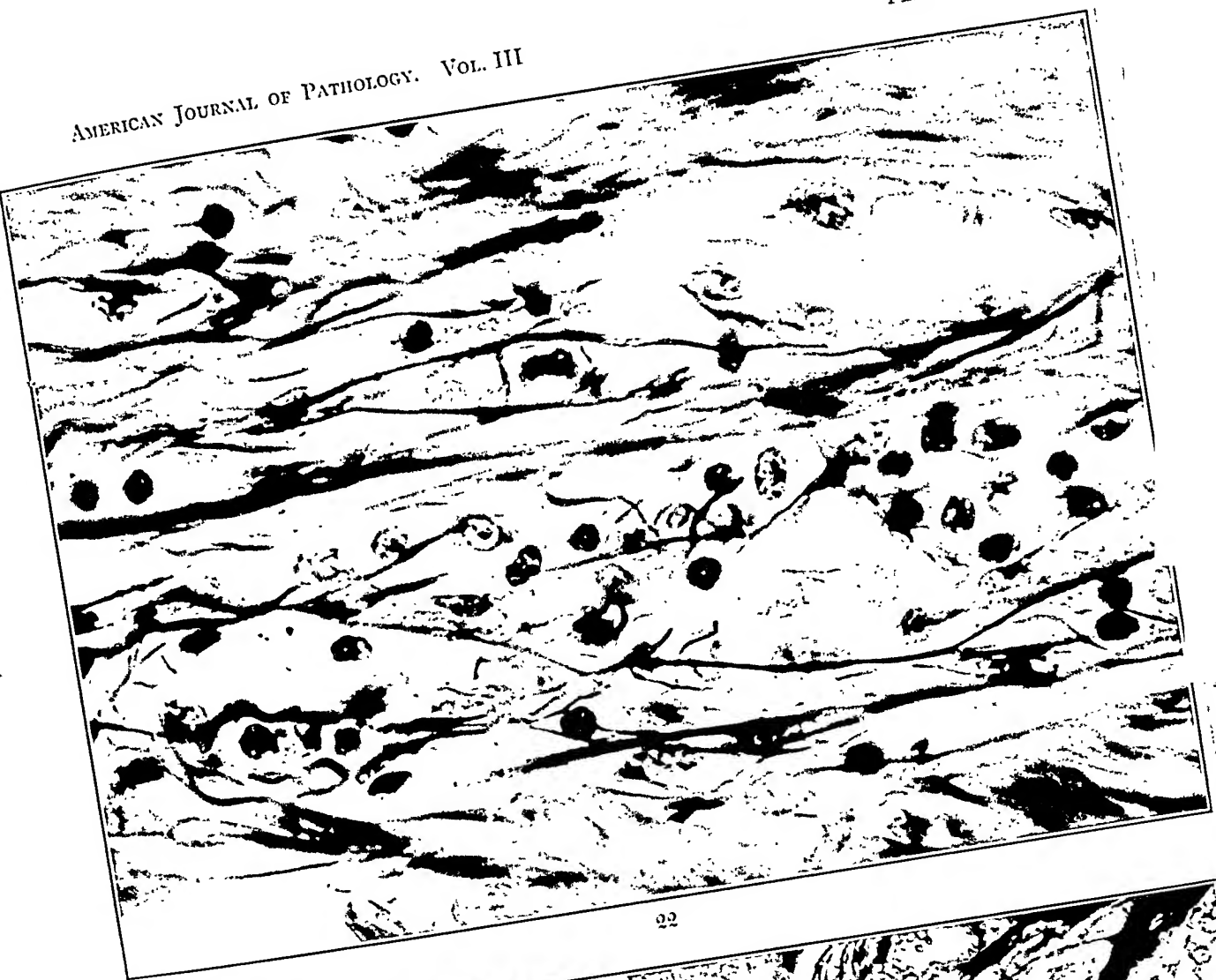
FIG. 23. Cancer of the breast stained by phosphomolybdic acid hematoxylin. All the collagen fibrils, both those that are separated and those that are compacted, stain deeply. $\times 250$.



20



21



22



23

Reticulum

Mallory and Parker

Lupu¹⁵ from an examination of fifteen cases of syphilitic aortic insufficiency concludes that the changes of the aortic valve in syphilis are due to a productive inflammation, and that the process spreads from the aorta through the commissure into the free margins of the leaflets, producing a marked thickening. He attributes the insufficiency to a shrinkage of the thickened margins of the cusps and shortening of their horizontal diameter.

Doehle⁵ states that syphilis of the aortic valve produces a thickening of the commissures and of the leaflets, which may be spread by the formation of a large hyaline plaque. He holds that the histologic picture shows no changes characteristic of syphilis.

Miloslavich¹⁷ explains the insufficiency of the aortic valve by thickened, dense and shrunken aortic leaflets.

Cabot⁶ mentions that syphilitic valve lesions are characterized grossly by a spreading of the commissure.

Karsner¹² states that the syphilitic process of the valve begins at the junctions of the leaflets, producing adhesions to the neighboring sinus walls and widening of the commissures.

This short survey shows that there is a divergence of opinion as to the nature of the involvement of the aortic valve in syphilis.

MATERIAL

Our study is based on necropsy findings of seventy-one cases. Blocks were made from various parts of the ascending and descending aorta and from different parts of the aortic valve. They were fixed in 10 per cent formalin and embedded in paraffin. The sections were cut 5 to 8 microns thick, and most of them stained with hematoxylin and eosin; others stained with Weigert's elastic tissue and Van Gieson's connective tissue stains. In three cases serial sections were made of the entire valve. Sections of the aortic leaflets and aortic wall of the sinus of Valsalva of ten cases of chronic non-syphilitic endocarditis of the aortic valve were used as controls, and in addition serial sections of a normal aortic valve.

GROSS FINDINGS

All cases show fusion between the lateral portion of the leaflets of the aortic valve and the aortic wall of the sinus of Valsalva. Normally the aortic leaflets are attached to one point of the aorta called the commissure. Fusion between the lateral part of the cusps and

THE INVOLVEMENT OF THE AORTIC VALVE IN SYPHILITIC AORTITIS *

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INTRODUCTION

Studies of syphilitic vascular disease have been numerous. The wealth of material at our disposal has made it seem wise to study the alterations which occur in the aortic valve leaflets in relation to the changes in the aorta.

Most text-books state that syphilis may involve the aortic valve from the aorta through the commissure, and explain the insufficiency as due to shortening and thickening of the valve leaflets together with a widened aortic ring which intensifies the incompetence. This is the view of MacCallum,¹⁶ who, however, admits that early stages have not been studied.

LITERATURE

Heller¹⁰ and Chiari⁷ in their classical description of luetic aortitis state that the syphilitic process may involve the aortic valve, causing insufficiency; and may involve the mouths of the coronaries, resulting in partial or complete occlusion. They, however, give no detailed description of the valve. Fabris⁹ states that syphilis of the valve starts in the region of the commissure. He pictures the lesions there as being fibrous and productive, but with no tendency to either fatty change, atheromatous change or calcification. He thinks that hyaline plaques at the site of the valve attachments may spread the commissures, causing insufficiency. Engel⁸ in a subsequent article expresses the same views as Fabris, but neither gives any details concerning the histology nor pathogenesis of the lesion.

Koch¹³ describes a severe case of syphilitic incompetence of the aortic valve. Three cusps were present, but the left leaflet was almost completely adherent to the aortic intima. Histologic examination revealed diffuse fibrosis of the cusp and fibrosis of the upper part of the intima of the aorta.

* Received for publication June 7, 1927.

proliferation of endothelial cells leading to a partial or even complete obliteration of their lumina. Some vessels are hardly recognized without the aid of an elastic tissue stain; others are identified by the presence of a few red blood cells in the lumen. The elastic fibers are often found split and increased in number. In spite of the elastic stain it is often impossible to determine whether the endothelial cells obliterating the lumen are intimal or adventitial in origin, a fact noted by Jakob¹¹ in his study of endarteritis obliterans of the brain vessels. Degenerative changes, however, of these proliferated cells are not observed. Since sections were taken from different parts of the aorta, it could be demonstrated that there are relatively more vasa vasorum and more pronounced obliterative changes nearer to the root of the aorta. This is striking in early cases, especially in the region of the commissure. In a number of sections taken from early cases, vessel changes are seen unaccompanied by round cell infiltrations. Old cases, however, show a marked new formation of connective tissue throughout the adventitia.

The media also shows perivascular round cell infiltrations about vasa vasorum. Here vessel changes are less frequent. The most prominent features, however, are broken elastic fibers and necrotic areas independent of lymphocytic infiltration. Early cases, which were the seat of a slight obliterative endarteritis of the adventitial vessels, show barely demonstrable lesions of the media.

In our early cases the inner portions of the media and the adjacent intima in those areas corresponding grossly to the commissures show changes very suggestive of mucoid degeneration. Numerous stellate cells are present, often characteristically triangular in shape with processes projecting in three directions. These cells are separated by a light bluish stained homogeneous intercellular material which gives a positive mucoid reaction (Bismark brown). No normal intima is seen in this region. Similar changes are found about the openings of the coronary arteries and the lateral portions of the cusps of the valves. The intima in other parts shows a slight increase in connective tissue and a few areas of hyalinization. There is a new formation of small sized vessels around the areas which show most marked degenerative changes. A slight infiltration of endothelial cells, lymphocytes and a few leucocytes, is found throughout, but chiefly located around the zones of mucoid degeneration.

the aorta naturally leads to a widening or separation of the commissures. Sixty cases show hyaline plaques of varying size in the region of the commissures. No relation could be established between the size of these plaques and the degree of separation of the commissures. The central portion of the free margin of the cusps in a vast majority of cases shows a marked thickening; eversion or rolling in twenty cases, and retraction in five cases only. In twenty-five instances the mouths of the coronaries are constricted. Twelve times both vessels are encroached upon; seven times the right and six times the left. In three cases the coronary openings are displaced.

In the majority of cases the most marked intimal changes of the aorta are found in a segment 4 cm. in length immediately above the aortic valve. Intimal thickening, marked hyalinization and sometimes calcification are present, and in addition, varying numbers of depressed scars and longitudinal wrinkles. In eight cases these changes are present only in the ascending aorta. The remainder of the cases show wider extensions with a distinct decrease in the severity of the changes in the arch and in the descending aorta. In a few cases, small atheromatous ulcers are observed. One case, a colored female 22 years of age who died of lobar pneumonia, shows only very few reddish, depressed, longitudinal lines above the commissures and no noteworthy intimal changes. This case is considered the earliest in our collection. The aortic valve shows marked adhesions between the leaflets and the aortic intima, in addition to the above-mentioned changes. The lesion was clinically silent. The ascending and descending portions of the aorta of our cases show, besides intimal changes, the wrinkles and puckering of syphilis, which are found more frequently in the ascending aorta and in the arch.

The hearts vary in weight from 300 gm. to 1000 gm., most of them being approximately 500 gm. The ages range from 22 years up to 72 years. The majority of patients are about 40 years of age. In twenty-one cases the interval between the primary lesions of syphilis and the first clinical signs of aortic insufficiency could be established. The most common interval is about 20 years.

HISTOLOGIC FINDINGS

Histologically, perivascular infiltrations of round cells are found in all sections, but more marked in the adventitia. The vasa vasorum of the adventitia, particularly in the early cases, show marked

vessels extend down to the base of the valve. This observation supports Aschoff's finding, namely that these vessels are branches of the vessels normally found in the base of the valve. No extension of vessels through the commissures is observed. Hyalinization and calcification are found frequently. The region of the commissure and the coronary openings show nothing unusual save for a varying amount of intimal thickening.

DISCUSSION

Our cases of syphilis show uniformly the widening of the commissures, which is pathognomonic of syphilis of the aortic valve. The common opinion is that the commissures are spread by the formation of hyaline plaques at the site of attachment of the leaflets to the aorta, extending wedge-shaped between the commissures. The mere fact that some of our cases show widening of the commissures without the formation of any hyalinized area in this region leads us to believe that there must be another explanation for the separation of the commissures.

The most constant histologic findings are endarteritis obliterans of the vasa vasorum and perivascular infiltration of lymphocytes in the adventitia. We have stated that our early cases show the endarteritis in a certain number of specimens without perivascular infiltration. Backhaus,² Warthin^{19, 20} and lately Waite¹⁸ hold that obliterative endarteritis of the vasa vasorum of the aortic adventitia is primary, a finding with which, in general, we agree. The perivascular infiltration also is found more frequently and more pronounced in the adventitia, as already mentioned by Larkin and Levi.¹⁴ We must assume that the endarteritis occurs in vessels of a certain caliber only, because the changes occur almost entirely in the adventitial vessels.

The media shows numerous necrotic areas independent of lymphocytic infiltration. For this reason we do not believe that these necrotic areas are always the result of a breaking down of the lymphocytic infiltrations. We are of the opinion that necrosis is a sequence of obliterative endarteritis of the vasa vasorum.

In the intima and inner parts of the media, in the region of the commissures, the mucoid degeneration is found. Warthin states that he was able to demonstrate spirochetes in hearts in areas which microscopically are mucoid in character. Chiari mentions in his

Early cases show only the mucoid degeneration and a few cellular elements, but no hyalinization.

The adherent parts of the leaflets show a new formation of small-sized vessels extending from the intima of the aorta through the commissures. Rather numerous fibroblasts and endothelial cells, some of which are spread by an edema-like material, are present in these parts, together with a few areas of hyalinization. The central portions of the cusps of early cases, which are not thickened grossly, show, in contrast to the lateral portion, no changes histologically.

Apparently in the older lesions the degenerative changes are less pronounced and chronic inflammation more evident. The lateral non-adherent portions of the leaflets of the valves in the older lesions show many new connective tissue cells separated by an edema-like, homogeneous material containing a few lymphocytes, plasma cells and endothelial cells. In examining the serial sections, it is evident that hyalinization and chronic inflammatory processes are more marked the closer to the adherent portion of the cusps the sections are taken. In the region of the commissure new vessels extend from the aorta into the adjacent parts of the valve. The largest amount of hyalin and the fewest number of cellular elements are observed here. The central portion of the valve leaflets, in cases which are grossly thickened, show only fibrosis, very few cells, but no vessels. The areas around the mouths of the coronaries in some cases show fibrosis associated with endothelial cells, plasma cells, lymphocytes and areas of hyalin. We could, however, always demonstrate a few areas of degenerative changes mentioned previously.

CONTROL

Our cases show grossly either adhesions between the leaflets (stenosis) or retraction of the cusps (insufficiency). None shows separation of commissures or adhesions between the cusps and aortic wall of the sinus of Valsalva. Six of ten cases show arteriosclerotic lesions in the ascending aorta, but in three instances the lesions are slight. The arteriosclerotic lesions consist of yellow areas of intimal thickening, areas of calcification and atheromatous ulcers. None of the cases shows any signs of syphilis.

Microscopically, numerous fibroblasts and a few endothelial cells are present in the valve leaflets. A new formation of vessels is observed in some cases. It could, however, be demonstrated that these

two regions which later may become hyalinized. The central portion of the cusps is not supplied by the vasa vasorum and therefore shows no characteristic syphilitic lesions.

The free margins of the central portions of the cusps are, in some cases, rolled and markedly thickened. No changes similar to those of the lateral portion are demonstrable. We believe that these areas of thickening are caused by the continuous mechanical pressure of the regurgitating blood associated with the insufficiency of the valve. The margins of the cusps in early cases are not thickened.

The changes around the mouths of the coronary arteries, also primarily degenerative in character, likewise undergo a chronic inflammatory reaction resulting in a new formation of connective tissue with hyalinization, with possible stenosis, or displacement of the coronary orifice.

CONCLUSIONS

1. The earliest lesion of syphilis of the aorta is an obliterative endarteritis of the vasa vasorum. The term "mesaortitis syphilitica" therefore gives an erroneous conception of the disease and "aortitis syphilitica" seems more appropriate.

2. Necrosis of the media is probably secondary and attributable to nutritional disturbances.

3. The changes of the aortic valve due to syphilis are characterized grossly by adhesions between the lateral parts of the leaflets and the aortic intima, leading to a separation of the commissures. The formation of hyaline plaques in this area is secondary.

4. Histologically the affected parts of the leaflets and the aortic wall of the sinus of Valsalva show first degenerative and later chronic inflammatory changes.

We are indebted to Professor H. T. Karsner for his helpful coöperation in this study.

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tables a myxomatous degeneration in the aorta, but gives no details. Very recently Brown and Pearce³ found myxomatous changes in the myocardium of syphilitic rabbit hearts. It must be assumed that the mucoid degeneration of the media and intima is primarily due to nutritional disturbances. While the media is supplied by the vasa vasorum alone, the intima and inner parts of the media are supplied by the circulating blood also. The latter prevents necrosis but is apparently insufficient to prevent degenerative changes. The degenerated areas become organized, as indicated by a new formation of vessels, endothelial cells, fibroblasts and young connective tissue. The perivascular infiltration of the newly formed vessels shows that the process does not undergo healing, but proceeds continually as chronic inflammation. In older cases, the number of cells in the newly formed connective tissue decreases and hyalinization occurs. The constant presence of the newly formed vessels prevents ulceration of these areas. The proliferative intimal changes with the formation of large hyaline plaques without ulceration are, in our opinion, directly referable to the syphilitic process of the adventitia and, as a rule, not to a coincidental arteriosclerosis. While arteriosclerosis often leads to ulcer formation in the intima, syphilis produces a productive inflammation without ulcer formation.

The most extensive lesions of the aorta are found in a segment of about 4 cm. above the aortic valve. As Spalteholz states, these areas show more vasa vasorum than any other region of the aorta. Syphilis is a primary disease of the vasa vasorum and therefore of greater significance in areas containing a large number of vasa vasorum. It appears in the vast majority of cases that syphilitic valve lesions occur only with involvement of this segment of the aorta.

The following hypothesis appears best to explain the separation of the commissure: The lateral portion of the aortic valve leaflets is, according to Bayne-Jones,⁴ supplied by the vasa vasorum from the aorta. The central parts of the free margins do not show vascularization. In the event of a nutritional disturbance only those areas supplied by the vessels in question will show degenerative changes. These are soon followed by a chronic inflammatory reaction. Organization of the inflammatory exudate of the affected parts of the leaflets and the corresponding area of the aortic intima of the sinus of Valsalva finally produces adhesions between these

DESCRIPTION OF PLATE

PLATE 143

- FIG. 1. Separation of the commissures and the involvement of the aorta in a segment of about 4 cm. in length.
- FIG. 2. Extensive adhesions of the middle leaflet to the sinus of Valsalva. The midportion of the leaflet only is free.

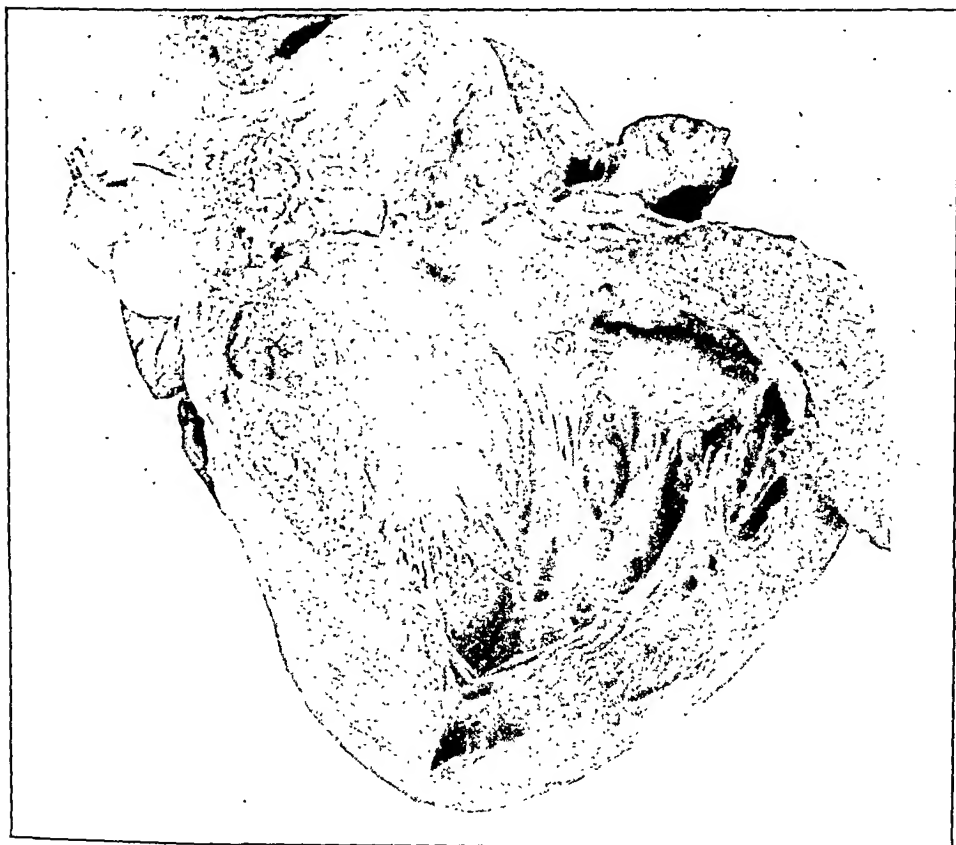
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PLATE 144

- FIG. 3. Separation of the commissure and adhesion of the lateral portion of the leaflet to the sinus of Valsalva.
- FIG. 4. Endarteritis obliterans of the vasa vasorum of the adventitia of one of the earliest cases.
- FIG. 5. Mucoid degeneration of the inner portions of the media and intima taken from the same case as the previous section.



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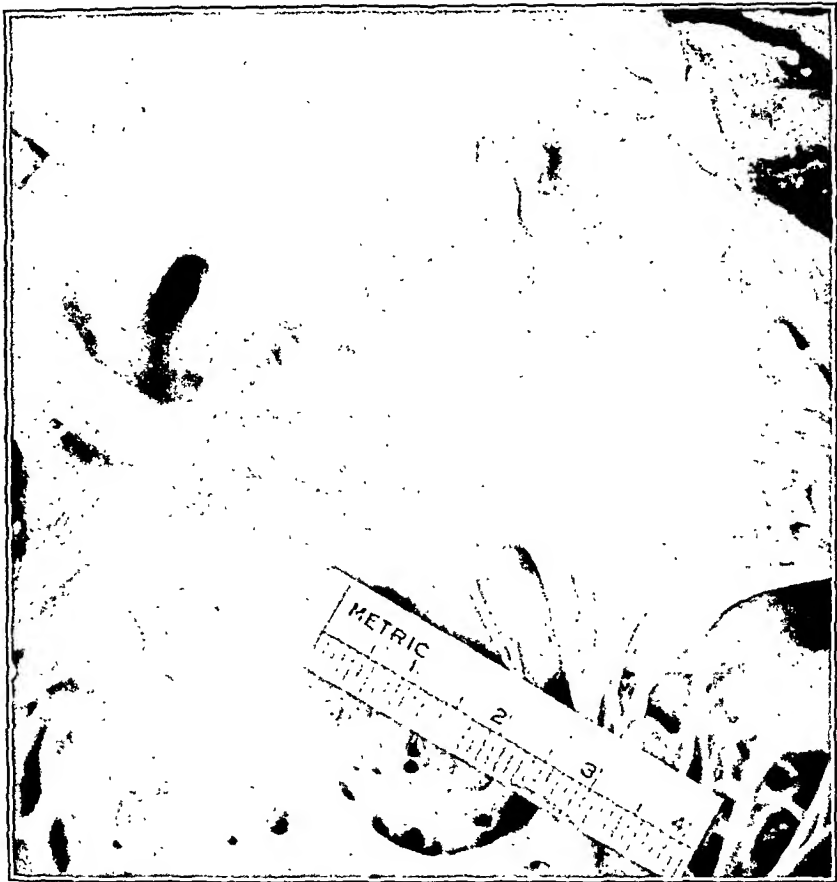


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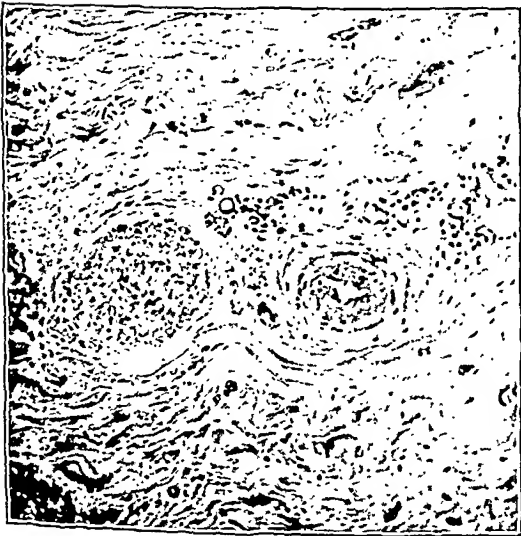
PLATE 145

FIG. 6. A section taken from the commissures. Note the new formation of vessels.

FIG. 7. An area similar to the previous figure showing a much later stage. Note the vessels and the perivascular infiltration of lymphocytes.



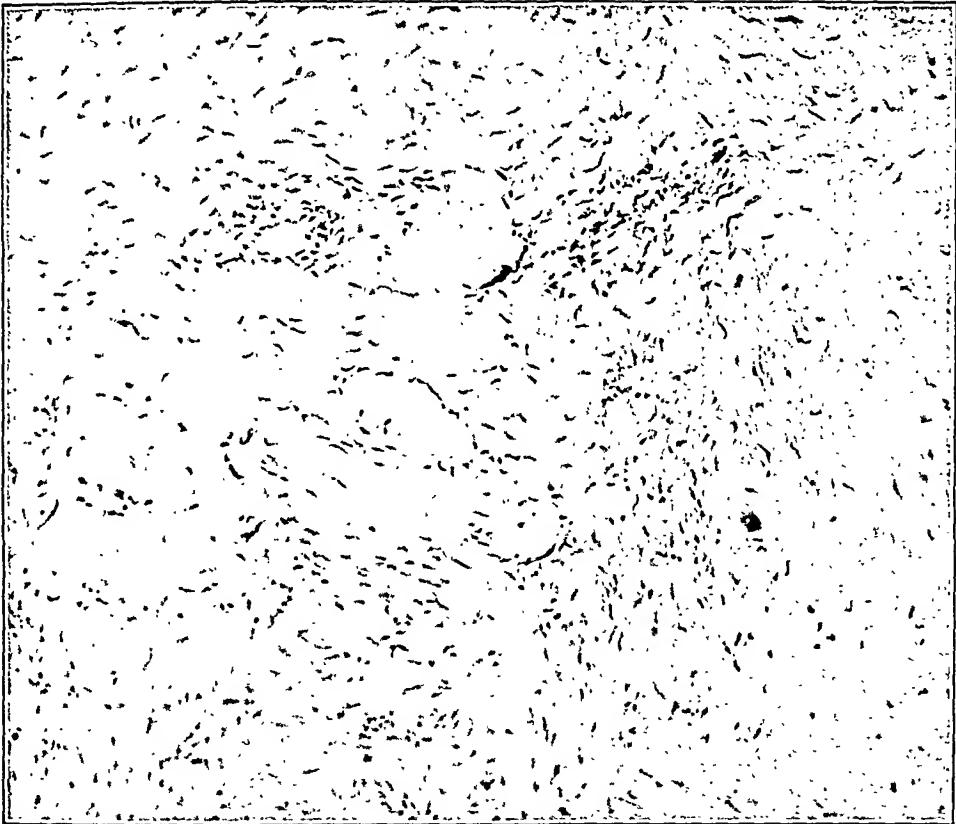
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SCIENTIFIC PROCEEDINGS OF THE
TWENTY-SEVENTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

ROCHESTER, NEW YORK
APRIL 15 AND 16, 1927

DERMAL TESTING AS AN AID TO DIAGNOSIS OF INTESTINAL PARASITIC INFESTATION. Matthew Brunner (by invitation), Albany, N. Y.

(Abstract not received.)

STUDIES OF ATTENUATION AND OF TOXIC PRODUCTION OF THE DIPHTHERIA BACILLUS. Mary W. Wheeler (by invitation), Albany, N. Y.

Abstract. These studies of attenuation of the diphtheria bacillus and its toxin production in synthetic media brought out certain interesting facts. One of six subcultures of the standard Park No. 8 strain of the diphtheria bacillus grew in Uschinsky's medium. This culture was avirulent and non-toxic and these properties could not be reestablished. Agglutination and absorption reactions alone gave evidence that the strain was closely related to the original Park No. 8 strain. More than fifty subcultures of the standard strain and thirty other strains failed to grow in Uschinsky's medium.

Growth and toxin production of the standard strain was obtained in synthetic media containing the chlorides, sulphates and phosphates of sodium, calcium and magnesium with dextrose as the source of energy and peptone as the source of nitrogen. No growth occurred if purified serum albumin or fibrin, pseudoglobulin, primary or secondary albumoses, purified peptone, asparagin, histidine or glycocoll were substituted for the peptone.

In the medium containing only peptone and inorganic constituents, a reaction of pH 7.4 to 7.6 and the presence of 0.2 per cent dextrose was most favorable for growth and toxin production. It was interesting to note that a potent toxin was produced only when the medium contained calcium and phosphate ions heated together in the presence of peptone. In the preparation of a medium with a peptone with high calcium content, the addition of other calcium salts was unnecessary. If, however, such a peptone was rendered free from calcium, it was necessary to add other calcium salts. The calcium ions could be replaced by equivalent amounts of barium or strontium, but not by magnesium or manganese. The addition of colloidal calcium phosphate to a medium which was unfavorable for toxin production, stimulated toxin production, but the potency of toxins from which the calcium was precipitated was unaltered. No significant differences were observed in the nitrogen metabolism of toxic and non-toxic filtrates of cultures of the standard strain grown in a medium of exactly the same chemical composition.

Discussion

(Dr. F. M. Huntoon, Glenolden, Pa.) I wish to congratulate Miss Wheeler on her paper. The production of diphtheria toxin has caused much difference of opinion because every producer has had his own ideas and theories particularly about the question of leaving the muscle-sugar in or removing it. It was felt that the production of diphtheria toxin was an art and each worker felt that his method was the only authentic method. Now Miss Wheeler has shown that it may be produced in peptone water if conditions are controlled. This work has aided very considerably the well-being of the diphtheria toxin products.

(Dr. I. S. Falk, Chicago.) I would like to ask how long it takes for the toxin to appear and the earliest stage at which potent toxin can be obtained.

(Dr. B. Steinberg, Toledo.) We had some experience in trying to produce soluble toxins and found that the nature of the media, the pH and the presence of sodium salts made a great deal of difference. This paper explains a good many things to me.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A COMPARATIVE STUDY OF BASIC BEEF HEART ANTIGENS IN THE WASSERMANN TEST. Marjorie C. Albray (by invitation) and L. W. Famulener, New York City.

Abstract. Eleven antigens were prepared by various recognized methods from each of a number of fresh beef hearts. Comparative tests, to determine (a) relative hemolytic activities; (b) anticomplementary properties and (c) antigenic values, were carried out for each series.

† The preliminary studies as reported were summarized as follows:

¶ (a) *Hemolytic bodies.* The Noguchi acetone-insoluble antigen prepared by the *old method* and the Kahn antigen showed the least amount of hemolytic bodies, the Kolmer antigen, a slightly higher content, while the Noguchi acetone-insoluble antigen prepared by the *new method* showed a higher content, but not to a marked degree. The simple alcoholic extract antigen showed quite a high content of hemolyzing bodies. The addition or omission of cholesterol did not noticeably influence the degree of hemolysis in parallel tests.

(b) *Anticomplementary substances.* The Noguchi acetone-insoluble antigen, prepared by the *old method* averaged a slightly higher content of these bodies than the Kolmer or the Kahn products. The simple alcoholic extract antigen showed the least amount, but perhaps this factor was masked by the higher content of hemolytic substances present.

(c) *Antigenic values.* In each instance, the Noguchi acetone-insoluble antigen, prepared by the *old* or the *new* methods, showed the highest values (unit strength). Both occasionally showed a higher value when cholesterinized. The Kolmer antigen approached the Noguchi antigens rather closely in some instances, but the average values were less. The Kahn antigens were somewhat lower in value and in general paralleled the cholesterol content when this was varied, but not always (one exception). In general, the Noguchi and the simple alcoholic extract antigens did not show any decided increase in antigenic values on the addition of cholesterol (0.2 per cent) although exceptions occurred.

These studies are being continued with the hope of further elucidation of the question.

(No discussion.)

THE ABSORPTION OF THE TOXIC SUBSTANCE OF NORMAL GOAT SERUM BY GUINEA PIG TISSUE. J. D. Aronson, Philadelphia, Pa.
(Abstract not received.)

LOCAL HYPERSENSITIVENESS. L. Dienes, Asheville, N. C.
(Abstract not received.)

STUDIES ON THE ABSORPTION OF UNALTERED PROTEIN. Matthew Walzer, New York City.
(Abstract not received.)

tion in an average period of three months. Their plasma contained more than 5000 neutralizing skin test doses of antitoxin per cubic centimeter. The use of this antitoxin in a series of cases of erysipelas was followed by a rapid decline in temperature, pulse and respiration, and disappearance and fading of the erysipelas lesions.

Discussion

(Dr. A. B. Wadsworth, Albany.) What was the virulence of the culture? You spoke of their being extremely virulent, how virulent?

(Dr. J. F. Anderson, closing.) I should have used the word toxic, extremely toxic, I judge by the toxin produced.

THE INCIDENCE OF VARIOUS SPECIES OF BACTERIA IN SPECIMENS OF SPINAL FLUID FROM CASES OF MENINGITIS. Ruth Gilbert and (by invitation) Marion B. Coleman, Albany, N. Y.

Abstract. In a bacteriologic study of 284 specimens of spinal fluid received since Jan. 1, 1920, from cases of meningitis occurring in New York State outside of New York City, only eighteen strains of meningococci were isolated. Three of these corresponded serologically to Gordon Type I, and three to Type II; one agglutinated equally well in Types II and III sera, and one in all three sera. The others were atypical strains.

The number of tuberculous spinal fluids was relatively very high, amounting to 167. Eighty-nine of the patients from whom these specimens were submitted were under 10 years of age.

Pfeiffer's bacilli were found in 23 cases, pneumococci in 33, and streptococci in 35; and in 8 instances organisms not commonly associated with meningitis were present.

It is important to note the relatively low incidence of meningococci in this series, which is even lower than that of *B. influenzae*. As the mortality in cases of meningitis due to *B. influenzae* is very high and there is some evidence that vaccine or serum treatment might be of value, a further study of such agents may be indicated.

Discussion

(Dr. H. Zinsser, Boston.) We have been studying immunization by the blood clot method during the last few years. The work that Dr. Lawson has done is very definite but not quite completed. I am rather surprised by the fact that the older studies of the influenza bacillus and the sub-groups do not seem to hold good in the studies we have made of the influenza bacillus which we have isolated during the last two years in Boston.

(Miss R. Gilbert, closing.) Those that we have isolated have not been definitely typed but we have most of them and the study can be continued.

SIPHONIC FERMENTATION TUBE FOR THE MORE RAPID ISOLATION OF THE COLON-TYPHOID GROUP OF ORGANISMS. F. B. Jones (by invitation), Montreal, Can.

Abstract. This siphonic tube was invented with the idea of reducing the time that is usually consumed in the growing and detection of these organisms. It consists of two glass bulbs which act as reservoirs and receive the fluid as it is displaced from the siphon by the collection of the gas produced during the proc-

(Dr. A. B. Wadsworth, Albany.) In addition to the fundamental observations on the conditions underlying toxin production, I think it is also interesting to point out, in this study of the attenuation of diphtheria bacilli, that the practically non-virulent strains could not be rehabilitated. Similar observations have been made on the pneumococcus with the attenuated strains that came from horses undergoing immunization. In the recent study of these strains, it was found that after a certain stage of attenuation the rehabilitation of the virulent type of culture is apparently extremely difficult, in fact, it was not accomplished in the experiments which were made.

(Dr. H. Zinsser, Boston.) I should like to ask Miss Wheeler whether in the attempt to rehabilitate the colony, her observations in any way corresponded to rough and smooth differences. This has just been discussed in the English Journal of Pathology. Has Miss Wheeler any observations which indicate that the non-virulent rough strains could be brought back to smooth? This is a condition which we have never succeeded in bringing about in our laboratory but which is quite commonly reported in the literature by others.

(Miss M. Wheeler, closing.) In reply to the question as to when the toxin appears: With the small quantities of medium used in most of these experiments, 15 cc., a potent toxin was present in from 24 to 48 hours.

At the time the experiments were made with the attenuated strain growing in Uchinsky's medium, no particular note was made as to whether this strain produced rough or smooth colonies. Colony formation, however, was not typical and I should say the colonies were rather hard and dry. Morphologically, the organisms were not typical but were very short and coccoid.

ACTIVE IMMUNIZATION AGAINST EXPERIMENTAL PERITONITIS. Bernhardt Steinberg and Harry Goldblatt, Cleveland, O.

Abstract. It has been shown in previous communications that following injection of colon bacilli suspended in physiologic salt solution, into the peritoneal cavity of dogs, these animals survived and at the same time showed a bacteremia. However, when colon bacilli were suspended in gum tragacanth and injected intraperitoneally the animals died but no bacteria were found in the circulation. It was postulated that death was due to absorption of toxic substances. The present communication is an indirect evidence of that assumption.

Dogs were immunized with small doses of living and killed colon bacilli. After varying intervals subsequent to the last immunizing dose, the dogs were given a lethal injection of gum tragacanth and colon bacilli. All the immunized animals survived.

Experimental peritonitis was produced by intraperitoneal injection of fecal material into dogs. A large number of dogs immunized with colon bacilli survived the injection of fecal material into the peritoneal cavity, while all the control non-immunized dogs died.

(No discussion.)

IMMUNIZATION OF HORSES TO ERYSIPELAS STREPTOCOCCUS TOXIN. John F. Anderson and (by invitation) George F. Leonard, New Brunswick, N. J.

Abstract. The results of the immunization of a group of horses with strains of erysipelas streptococci isolated by Dr. Konrad E. Birkhaug are reported. Using a combination of the injection of toxin from these strains of streptococci and the blood clot method of immunization, the horses have been bled for produc-

The clue perhaps to the method of study is contained in the rather numerous papers that have been written on the endothelial leucocyte or histiocyte.

(Dr. Wolbach.) Don't you think it would be advisable to advise a uniform technic? For your Council to state which vital stain or supra-vital stain would be best?

(Dr. Callender.) I do not think we are prepared to establish a uniform technic for vital staining but would refer that question to the Committee, Drs. Mallory and Ewing, for reply.

(Dr. James Ewing, New York.) Any good staining methods are all right.

(Dr. A. Plaut, New York.) Dr. Callender raised the question of erythroblastoma. Ribbert has published one case. Diffuse erythroblastosis seems to occur only in the newborn and I wonder if it belongs directly to the diseases which have been grouped here. Congenital erythroblastosis is an extreme exaggeration of the red blood picture present in the newborn. It is connected either with jaundice or severe congenital hydrops.

(Dr. Callender.) It was not my intention to call that a tumor, but just to point it out.

(Dr. Plaut.) In speaking about tumors, I only referred to Ribbert's case.

(Dr. Ewing.) I have been over all these cases and I am convinced that the registry is of value. I have learned a great deal from the forty or fifty cases. One thing I think highly important and that is, so far as possible, to have the bacteriologic studies. Several of these cases were very important bacteriologically. As for stains, every man has his own specialties in this field, and well fixed material stained by any method that is capable of analysis is the essential thing. Many of the specimens were not very well fixed. Bacteriologic studies and very thorough fixation of tissues seem the most important desiderata.

EXPERIMENTS OF TWO YEARS ON THE RELATION OF HODGKIN'S DISEASE AND THE ORGANISM ISOLATED BY GRUMBACH. Herbert Fox, Philadelphia, Pa.

Abstract. Some observations upon the relationship of diphtheroids to Hodgkin's disease and their pathogenicity, the occurrence of the lymphogranuloma in the lower mammals especially primates, and the blood picture of rhesus macacs are reported.

Repeated injections of the diphtheroid isolated by Grumbach from the blood stream of Hodgkin's disease during its active phase, into non-tuberculous rhesus macacs, during two years failed to produce in these animals any lesion that, grossly or minutely, resembled Hodgkin's disease. Injection of these cultures into guinea pigs confirmed in part Grumbach's work but it does not seem to the writer that the lesion in the lungs or lymphatic tissue should be called lymphogranuloma. With the monkeys the method was based upon the repeated introduction of large numbers of the bacteria so that constant assault upon the lymphatic tissue would exist. That the organisms were present in the body of the monkeys was indicated by the fact that No. 2 animal when sacrificed two months after the last dose, had the bacteria in the heart's blood.

Observations of the two animals during two years, and of the tissues, blood and serum of one of them at death fails to reveal any specific pathology indicating pathogenicity of the diphtheroid. The only and important specific reaction was a small amount of agglutinin. No complement fixation amboceptor appeared.

Hodgkin's disease has not been observed in the 3,000 mammals and 5,000

ess of fermentation of the sugar. These bulbs are connected by an intervening siphon which consists of glass tubing of small caliber and is bent in the form of an inverted "V." As the gas is produced it readily collects in the acute angle of this inverted "V" and breaks off the direct communication, through the fluid, between the two glass bulbs.

This tube may be used as a fermentation tube generally wherever it may be found necessary to use such a tube.

(No discussion.)

THE FIRST ANNUAL REPORT OF THE REGISTRY OF TUMORS OF THE LYMPHATIC SYSTEM. G. R. Callender and J. F. Coupal, Washington, D. C.

Abstract. Forty-two tumors tentatively classified as shown in the following table have been received by the registry.

Tumor Registry, 1927

Tuberculosis		1
Hyperplasia in Lymph Nodes, Generalized		2
Lymphosarcoma { Plasma cell type.....	1	4
{ With terminal leukemia	1	
{ Typical	2	
Reticulum cell, malignant { Localized.....	1	6
{ Diffuse.....	5	
"Hodgkin's Disease" { Typical	6	14
{ Mixed cell.....	1	
{ Sclerosing	5	
{ Large cell	2	
Lymphatic Leukemia		7
Chloroma { Lymphatic Leukemia	1	2
{ Myelogenous Leukemia	1	
Hyperplasia, Erythrocytic tissue (Infant)		1
Carcinoma { Lung	3	5
{ Testicle	1	
{ Squamous cell.....	1	
TOTAL		42

A classification of these tumors based on histogenesis is presented for discussion. A schematic outline of this classification is shown below. Pathologists were urged to send in samples of tumors of this kind for registration wherever it was possible for them to get the necessary data together, without reference to the person referring the case, it being understood that such cases were only available for study, and would not be reported without the consent of the pathologist registering them.

Discussion

(Dr. S. B. Wolbach, Boston.) I would like to ask Dr. Callender which vital stains he would like employed.

(Dr. Callender.) As I have done no work with the vital stains in this group of tumors I am not competent to suggest just which of the dyes should be used in the study. Janus green and brilliant cresyl blue have both been used, the former immediately on removal and the latter by injection before operation.

phile picture and reaction to radiation. These are separate from Hodgkin's disease which varies with its type and from endothelioma and reticulum cell sarcoma. These features, if corroborated with a biopsy, will somewhat simplify the classification of a given case.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) I think it well worth while to emphasize the normal variations in the blood picture of any animal. Probably this is more noticeable in the monkey than in other laboratory animals. However, I feel that that should not prevent an attempt to draw deductions from hematologic studies if extra precautions are taken to standardize the counts in every way possible. If the same individual use the same pipette at the same time of day under conditions that are practically constant, many of these fluctuations will be minimized to a point where the results will be useful.

(Dr. B. Steinberg, Toledo.) A few weeks ago I had the opportunity to run through a series of twelve dogs. We had taken the temperature at two hour intervals, leucocyte counts, etc., and it was surprising to find how they varied. The temperature varied from 96° F. to 100° F. and the leucocytes from 4,000 to 12,000 in apparently normal dogs. We therefore could not find any normal standard to make out further experiments because of this variation in temperature and leucocyte counts.

(Dr. James Ewing, New York.) We have in our museum a uterus which was removed some years ago from a dog. The endometrium is about one-half an inch thick. I called this Hodgkin's disease but whether genuine or not I do not know. It has the histology and the gross anatomy of Hodgkin's disease.

(Dr. H. Zinsser, Boston.) I would like to say that in the case of rabbits a sort of fluctuation of the leucocyte count and the temperature appears but if one follows it consistently for long periods one can get departures from the normal. As far as diphtheroids of Hodgkin's or of any other disease, I do not think any bacteriologist who is familiar with diphtheroids pays any attention to an etiologic claim for a diphtheroid unless there is absolutely overwhelming proof.

(Dr. J. F. Coupal, Washington.) This paper brings out quite prominently the importance of quantitative studies of radiation in the Registry records as well as the value of the white counts. We do not need to annoy the clinicians too greatly about the history as long as we get the quantitative and qualitative record of the amount of X-ray and radium used.

(Dr. E. B. Krumbhaar, Philadelphia.) It would be interesting to know where significant changes had taken place and whether the polymorphonuclears in the young or old forms had been increased or decreased. I might take this opportunity to urge that a slight modification of the differential count will permit one to divide the polymorphonuclears into the old and young forms by a consideration of the nucleus and a very occasional metamyelocyte, so that the time for doing the differential count is only added to by five or ten minutes. In one case of low grade chronic lymphatic leukemia I made for several weeks an Arneith's formula and there appeared a constant reduction, the curve shifting rather to the right.

INTRA-VITAL STAINING AND PHAGOCYTOSIS IN HODGKIN'S DISEASE, LEUKEMIA AND SARCOMA. Herbert Fox, Philadelphia, Pa.

(Abstract not received.)

birds at this laboratory. Study of the lymphatic and myeloid tissues of primates indicates that the anatomic basis for the disease is as available as in man. Examination from an etiologic standpoint leads nowhere because it begins nowhere. The cases in domestic animals as reported in the literature are not convincing and are usually sarcoma or leukemia. The more acceptable cases might be myeloid hyperplasias dependent upon anemia.

It is not safe at present to attempt to state a normal blood count of the rhesus macac. There are variations due to factors not yet understood. It is best to determine the normal variations of each experimental macac and establish his own modes. Hemoglobin and red cells vary with the health of the beast and tend to rise after the animal comes from the dealers and becomes accustomed to new surroundings and gets good food. Repeated handling may affect the blood count; it certainly makes the temperature higher.

Leucocytes vary from 5,000 to 25,000 with an average of 10,000 to 13,000. Neutrophils are the most numerous individual cell, having a percentage average of 44 to 54 per cent. Lymphocytes are next, their average being 33 to 47 per cent. Eosinophils and basophils are extremely variable and this work revealed no mode nor any reason for the variation. Many cells comparable to neutrophilic polynuclears have fine basophilic granulations. Mononuclears average 5 to 6 per cent. The leucocytes are not greatly affected by food. Sometimes the number drops, sometimes rises, after a meal following a fast.

The leucocytes of rhesus macacs perform a daily cycle of numbers with a high peak in the late evening hours. The course during the day hours is irregular but fails to show a distinct rhythm. The lowest counts seem to be from 11 A.M. to 3 P.M. The late evening rise is due chiefly to polynuclears. The leucocytes rise in numbers while the temperature is performing its normal night drop. This daily rhythm occurs without relation to food or sleep.

(Discussed with next paper.)

RELATION OF POLYNUCLEAR NEUTROPHILES AND THEIR REACTION TO X-RAY, TO CERTAIN LYMPHADENOPATHIES. Herbert Fox, Philadelphia, Pa.

Abstract. From the evidence here collected the leukemic group is composed of true chronic lymphatic leukemia, sublymphatic leukemia and leucosarcoma in that they have, with lymphocytosis, a low number of neutrophils which are very susceptible to radiation treatment. Aleukemic lymphadenosis tends to have the percentages and numbers of neutrophils and lymphocytes approach normal with relative insusceptibility to radiation. The cases have a tendency to caseous involvement, which is, however, not in our two cases attended by polynucleosis, and therefore suggest myelomas which are not characterized by a peculiar blood picture. This group of adenoses resembles somewhat in hematology lymphosarcomas. The tendency to final malignant stages as evidenced by the bony tumors in adenosis, increases the resemblance. The neutrophils in lymphosarcoma do not fall consistently under radiation.

Hodgkin's disease is marked by a polynucleosis which is indifferent to radiation except in the malignant and febrile cases when these cells are quite variable. Our single cases of reticulum cell sarcoma and endothelioma had neutrophils unaffected by radiation and of no definite numbers.

From these figures then, one can deduce that there is a leukemic group—true chronic leukemia, sublymphatic leukemia and leucosarcoma, and a sarcoma group—aleukemic lymphadenosis and lymphosarcoma, with distinct neutro-

whole or in part. In a few were central areas of the same consistence as the rest of the node, but of canary yellow color. None was necrotic.

Spleen: $23 \times 15 \times 10$ cm. Surface nodular. On section there were numerous white, gray or yellowish circumscribed nodules scattered through the splenic substance. These nodules appeared to be of the same type as the enlarged nodes. The largest nodule was 2.0 cm. in diameter.

Liver: Enlarged. Weight 2,660 gm. There were numerous white, circumscribed areas similar to those in the spleen, but smaller.

Bone marrow (rib): Hyperplastic, grayish red.

Microscopic Examination: The lesions in the lymph nodes, spleen and liver are of two types: (1) Diffuse lymphoid hyperplasia and infiltration. (2) Multiple nodular and diffuse neoplasia of reticulum cells.

The first lesion is identical with that usually found in lymphatic leukemia.

The second lesion is in the form of large mononuclear, occasionally multinuclear cells of varying size, shape and arrangement. In some sections the arrangement is that of a sarcoma composed of fairly uniform cells; in others, the presence of giant cells causes a superficial resemblance to Hodgkin's disease. However, there is no necrosis, fibrosis, or eosinophilic infiltration.

Both lesions are present in the liver, spleen and all groups of lymph nodes examined. The lesions of leukemia and of tumor may be seen side by side, often with intermingling of their cells. The cells of each, however, maintain morphologic independence.

That the tumor is composed of connective tissue cells is evident, but the cell of origin is difficult to determine. However, a study of the sections indicates that the cells probably arise from the reticular cells of the lymphoid tissues; and there these cells form the boundary of lymphatic sinuses, from reticulo-endothelium, although they do not actively produce reticulum fibers.

The bone marrow shows replacement of the marrow cells by diffuse lymphoid tissue.

Discussion. The association of leukemia with a tumor suggests several possibilities of etiologic relationship. This is particularly true of this case because one of the lesions (the tumor) apparently arises from reticular and reticulo-endothelial cells, which are thought to be potentially capable of producing blood cells of various types.

However, the cells of the tumor and of the leukemia, although closely intermingled, remain morphologically distinct, without transitions from one cell type to the other.

That the lymphoid lesion is a true leukemia, and not a "leukemoid reaction" to the presence of a tumor, is indicated by the typical histologic lesions of leukemia in the organs. Likewise, there is no direct evidence pointing to the dependence of the tumor on the presence of leukemia.

The lesions in this case can best be explained by regarding them as separate conditions which, although closely associated, are morphologically distinct.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) I believe that Dr. Richter's neoplasm belongs to the same group as those that we have seen in our material and have called leukemic sarcomatous Hodgkin's or leukemic reticuloblastomas. Of twenty-nine cases of sarcomatous Hodgkin's twelve developed a leukemic transformation. These neoplasms are all genetically related. They all belong to the

ACUTE LEUKEMIA WITH EXTRA MEDULLARY MYELOID CENTERS. Harry T. Marshall and (by invitation) Katherine Woodward, University, Va.

Abstract. A negro child of five, after acute infections repeated over three months, died with features of acute leukemia and pronounced anemia. Pneumococcus on blood culture at necropsy.

The kidneys showed nodules, bright red in color like infarcts. Similar red splotches on dura mater. Malpighian hyperplasia in spleen. Pneumonia, organizing. No general glandular enlargement. Pansinusitis.

Microscopically, the red splotches in dura and kidney were not infarcts, hemorrhages or leukemic infiltrations. They were myeloid centers. Similar changes appeared in lung and to a less degree in peribronchial lymph nodes.

Conclusions are drawn as to the scope of the term "acute leukemia"; its applicability to this case, and its nature. The relation of findings to the definition of lymphocytes is indicated. Suggestions are made as to the bearing of this case upon current views of blood formation.

NOTE: Dr. Marshall defined tumor and leukemia before beginning his paper.

Discussion

(Dr. James Ewing, New York.) Did you find any traces of hemoglobin in these cells?

(Dr. Marshall.) Oh, yes.

(Dr. Ewing.) You didn't mention it.

(Dr. Marshall.) In the early picture the cells had distinct hemoglobin at all stages of hemoglobin formation from a deep basic cytoplasm, to the cells in the kidney interstices and lung interstices with various stages of hemoglobin.

(Dr. A. S. Warthin, Ann Arbor.) Did you examine the periosteum for similar cells?

(Dr. Marshall, closing.) No.

GENERALIZED RETICULAR-CELL SARCOMA OF LYMPH NODES, ASSOCIATED WITH LYMPHATIC LEUKEMIA. Maurice N. Richter, New York City.

Abstract. The purpose of this communication is to report a case in which a generalized tumor of unusual type, apparently primary in the lymphatic system, was associated with the clinical, hematologic, and histopathologic features of lymphatic leukemia.

The patient was a man, 46 years of age, with marked enlargement of the superficial lymph nodes, spleen and liver. The duration of the disease was said to be seven weeks.

Blood examination showed 98,400 white cells per c.mm., of which 90 per cent were lymphocytes, mostly of the small variety.

Death occurred three weeks after admission. The clinical diagnosis was lymphatic leukemia.

Necropsy: B. H., No. 11643. Summary of positive findings.

Generalized lymphadenopathy. The abdominal nodes formed a retroperitoneal mass which extended from diaphragm to pelvis, and from spleen to right kidney. Cervical, axillary, inguinal, epitrochlear, mesenteric and thoracic nodes were also enlarged. The nodes were discrete, soft and measured from 0.1 to 8.0 cm. in diameter. Some were white or gray, some hemorrhagic in

Discussion

(Dr. James Ewing, New York.) I would like to ask Dr. Warthin whether he thinks this is the same tumor condition.

(Dr. A. S. Warthin, Ann Arbor.) I do not think so.

(Dr. A. Plaut, New York.) I would like to ask how much fibrosis there was. One slide showed extensive fibrosis. Were there many spots like that?

(Dr. Schultz.) The section shown was from the periphery. There was not only no increase but a condensation of the bands of fibrous tissue near the surface.

(Dr. Plaut.) I asked for a special reason, because there was a condition of a tumor mass arising in the abdomen that was an "inflammatory tumor of the omentum," which Ewing has referred to as pseudosarcoma. A few years ago a case came into my hands and I found a large number of small foci of typical lymphoid tissue scattered throughout the extremely fibrosed omentum. There might be something similar in Dr. Schultz' case with the lymphoid tissue outgrowing the fibrous masses in the omentum. There must be some irritation but what kind of irritation seems to make little difference. This is only a suggestion.

(Dr. Schultz, closing.) In reply to Dr. Plaut I can only say that the striking thing was the diffuseness of the process; there were no nodules, but the entire mesentery and omentum were composed of a very cellular tissue of the structure of lymphadenoid tissue. There was no proliferative reaction whatever on the part of the stroma, and the lesion has none of the characters of a granuloma.

MALIGNANT LYMPH FOLLICLE HYPERPLASIA OF SPLEEN AND LYMPH NODES.

George Baehr and (by invitation) Nathan Rosenthal, New York City.

Abstract. A report of a study of six cases which represent a distinctive clinical and pathologic entity that can be differentiated from ordinary lymphosarcoma. The salient characteristics include: (1) lymphadenopathy due to hyperplasia of the germinal centers of the lymph follicles; (2) splenomegaly due chiefly to enormous enlargement of malpighian bodies, the weight of the spleen increasing up to 1,800 grams; (3) absence of abnormal cells in the blood; (4) absence of anemia or cachexia; (5) tendency to development of serous effusions in the pleural and peritoneal cavities due to the pressure of mediastinal or abdominal lymph nodes upon venous or lymph vessels; (6) absence of involvement of tonsils and lymphatic apparatus of the gastro-intestinal tract; (7) tendency to lymphatic infiltration in lacrymal gland resulting in unilateral exophthalmos. Aside from the absence of anemia and cachexia, the chief differential feature distinguishing the condition from lymphosarcoma is its origin multicentrically throughout the body in the lymph follicles, whereas lymphosarcoma arises monocentrically and spreads by lymphatic extension. Both lymph node and splenic enlargements respond with remarkable promptness to X-ray or radium therapy.

Discussion

(Dr. G. R. Callender, Washington.) We have two cases quite similar in the registry, one of which we followed for six years. Sometimes an overdose of X-ray gives rise to serious clinical symptoms.

(Dr. Baehr, closing.) I shall be glad to accept the correction as to terminology for there are undoubtedly more follicles in the spleen than are normally present,

same group as far as their pathogenesis is concerned and represent different degrees of differentiation.

(Dr. J. F. Coupal, Washington.) We have taken refuge in the Registry of the museum by looking upon the blood, not as a tissue like some of the older histologists did, but as an organ which helps in understanding the cellular pictures found in this type of case.

(Dr. G. R. Callender, Washington.) I hope nobody who has a case approaching this in interest will avoid the Registry.

(Dr. A. M. Pappenheimer, New York.) I had the privilege of studying these sections. On looking up our material at the Presbyterian Hospital for similar cases, we found five which showed an histology identical with the tumor part of Dr. Richter's case. None of our cases, however, was associated with a leukemic condition of the blood. This might indicate with Dr. Richter that the two processes are distinct.

(Dr. George Bachr, New York.) I should like to lay emphasis upon the fact that chronic lymphatic leukemia is often a very chronic disease which exists throughout a large part of the patient's lifetime. In such circumstances it may occasionally happen that the individual will develop some other type of neoplastic disease. We have observed two cases of primary carcinoma of the lung in patients with chronic lymphatic leukemia.

(Dr. M. N. Richter, closing.) With regard to the diagnosis of Hodgkin's in this case I do not think the section is typical of the usual type of Hodgkin's that we see, although the eosinophils are not essential. In examining a good many nodes from all parts of the body, and nodules from the spleen, liver and other organs I might hope to find an eosinophil occasionally, but I was unable to do so in any tissue examined. Another thing of importance is definitions of the various conditions that we are talking about. As we must rely on the histology for the diagnosis, we ought to know what we mean by the clinical terms "tumor" and "leukemia." I fail to find very good definitions of these conditions, particularly of tumors and I do not know how to tell whether leukemia is or is not a tumor without knowing what a tumor is.

DIFFUSE LYMPHOMA OF MESENTERY ASSOCIATED WITH CARCINOMA OF COLON.

Oscar T. Schultz, Chicago, Ill.

Abstract. In a man aged 54 years a mass was palpable in the left upper quadrant of the abdomen. The condition was believed to be an inoperable retroperitoneal sarcoma. Roentgen therapy was instituted, but had no detectable effect. Seventeen weeks after this admission symptoms of acute intestinal obstruction developed very suddenly. Colostomy was done. Death resulted from peritonitis three days later. At necropsy the entire mesentery was transformed into a layer of pale tissue 3 to 5 cm. thick, the liver and omentum were studded with carcinoma metastases, and an obstructing annular carcinoma was situated at the splenic flexure of the colon. The mass palpable during life was a portion of the thickened mesentery. The latter was free from carcinoma but had been transformed into a cellular tissue composed of closely placed lymphocytes. In places a follicular arrangement was present.

(Dr. Zinsser.) I think it is almost my duty to ask Dr. Mallory whether he has anything to say.

(Dr. F. B. Mallory, Boston.) I shall be sufficiently destructive in the next paper so I shall refrain now.

(Dr. H. T. Marshall, University, Va.) In regard to one or two of these pictures that were shown which resemble the picture that I found in the infant, I was able to see indications of actual activity in the elongated cells between the renal epithelium, not within the tumor but in the new growth occurring between, which would not be tumor in the sense described by Dr. Warthin. Moreover, like Dr. Ewing I feel that Dr. Warthin has failed to take account perhaps of the adaptive changes that lymph nodes are capable of exhibiting. The only way to get any result would seem to me to be a tabulated series, studying the known irritants in measurable gradients. I think you will find that many of the changes are reactions in the nature of inflammatory adaptation.

(Dr. M. N. Richter, New York.) Now that we have definitions of the conditions which we are discussing I think perhaps we are in a position to talk of the etiology and the pathogenesis. I am particularly interested in the various blood pictures resembling leukemia. We know that various tumors metastasizing to bone marrow might throw out bone marrow cells into the circulation. I wonder whether the metastases to lymph nodes would not throw out various lymphocytes by a mechanical process without the necessity of assuming a separate neoplasm for their appearance in the blood. I would like to ask, in tumors associated with myeloid leukemia, what picture the bone marrow showed. Whether, for example, Hodgkin's in the bone marrow might possibly account for the irritation and the outpouring of myeloid cells.

(Dr. J. F. Coupal, Washington.) I think if we reiterate that these tumors arise from the various portions of the reticulo-endothelial system considered rather as an organ than as a tissue, we can justify all of Dr. Warthin's paper. If we remember that their stages of reversion proceed back to the primitive form, the embryoma, we can understand why these tumors exhibit so many cellular variations. The tendency for these tumors to form after chronic inflammation of this system, especially in its grosser lymphatics, indicates that it is subsequent to hyperplasia and hyperfunction rather than irritation and structural defects usually the cause of tumors in other organs. In addition, this system has repeated calls for a massive increase of physiologic action, as the rapid formation of large numbers of leucocytes and the vast increase in function shown in swelling of the lymphatics consequent upon infection. These marked variations of amplitude of function correspond to the marked, periodical, physiologic variations that occur in the breast and uterus, and which expose them to their high incidence of tumor.

(Dr. Zinsser.) I venture into this discussion reluctantly but I have been rather surprised by the frequent reference to the infectious origin which has been mentioned. This in particular applies to Dr. Ewing's reference in regard to Hodgkin's disease. I have been associated with some of the studies indirectly with Dr. W. C. Clark in which two chimpanzees observed by complement fixation reactions and bacteriologic studies were innoculated directly in the upper arm with Hodgkin's material under accurate control for many months. These experiments were completely negative. As a bacteriologist, I no longer have any confidence in the infectious origin of Hodgkin's disease.

(Dr. A. S. Warthin, closing.) I should like to lay out my old friend Ewing completely but I have great hopes for him. He will be something more than a

but the malpighian bodies which normally are present are tremendously hyperplastic and although small collections of lymphocytes may have developed into additional follicles, I am at a loss to know what else one could call the condition except malignant lymph follicle hyperplasia. As to the caution about use of the X-ray, we have also records of a case which was treated with radium by someone else. The treatment was kept up long after the evidence of lymphatic involvement had disappeared, until eventually the patient developed a profound anemia and other evidences of radium poisoning.

THE NEOPLASTIC RELATIONSHIP OF HODGKIN'S DISEASE, ALEUKEMIC AND LEUKEMIC LYMPHOBLASTOMAS AND MYCOSIS FUNGOIDES. Alfred S. Warthin, Ann Arbor, Mich.

Abstract (not received).

Discussion

(Dr. H. Zinsser, Boston.) I wonder if Dr. Warthin knows how that sounds to a bacteriologist. It sounds like a catalogue of the ships in the second book of Homer. Perhaps Dr. Ewing may have some comments.

(Dr. James Ewing, New York.) I remember, Dr. Warthin, that paper which you presented in the early nineties. My criticism of the conclusions, I think, on the whole, was justified by the manner in which you yourself, just now, tore the entire classification to pieces. The only thing you left was the first term in the classification, "lymphoblastoma" and that I would demolish, as you do not seem to have the courage to do, by expressing the belief that there is no such thing as a parent cell of all these various neoplasms. I confess that Dr. Warthin's enormous material, which he has worked up for so many years with great care, may justify him in his rather firm and dogmatic opinions. However, I think Dr. Mallory could duplicate the numbers and possibly many of the varieties. Modesty prevents me from telling what we might show at Bellevue and New York and I think that Chicago could go a long way, so that on the whole one might encounter equally dogmatic opinions drawn from almost the same amount of material in many cities. This encourages me to retain some of my own notions about the subject which are not nearly as clear or as positive as Dr. Warthin's. The main ground for dissatisfaction with his Table is that in 1897 he proposed one name for this entire group of lymphoid reactions and he holds that opinion still. I think that is not going to help us much. I believe that as long as the etiologic factor or factors are missing, either bacterial, toxic or neoplastic, the only way of making real progress is to adhere closely to the morphologic distinctions in the hope that some day we shall find different specific agents associated with these pictures. It is my firm belief that that will be the outcome. I do not believe it is wise to assume that all leukemic processes are neoplastic or spontaneous non-altruistic growths. I believe many of them have distinct relations to infections. I have seen leukemic processes develop from pneumonia, acute tonsillitis and diphtheria and run a short and fatal course, or run a longer course into chronic leukemia. I think it entirely different from Hodgkin's disease which to my mind bears all the marks of a specific infectious disease. Although we may not be able to find clear differences in all cases, how are we going to make progress by throwing them all into one category? I would rather see the most minute differences emphasized and a classification based upon them until the time when the etiologic factors unify or subdivide the entire group.

Discussion

(Dr. A. Plaut, New York.) I would like to ask Dr. Weller and others as well, whether from this picture there was created an impression of a true neoplasm. I have the impression that these are not neoplasms. They are not circumscribed, not adenomyoma, but diffuse lesions. These glandular ducts which we have seen look really perfectly normal. They do not show signs of autonomous growth and the idea that such tissue should metastasize, I do not think, would occur to any of us in looking at slides of this character. As to the question of origin, I personally do not favor the theory of Sampson but rather the peritoneal theory which, I think, in some cases has been proven. But there is another possibility to be considered; these glandular ducts might be transformed lymph vessels, these epithelial cells might originate from endothelial cells. I fully realize what a shock this might cause to some tissue workers. I have seen a number of slides which led me to think of this possibility. Later I saw the excellent photomicrographs in the paper by Schiller in Vienna who comes to the same conclusion. If this theory is true, it can help us in understanding the diffuse multicentral character of adenomyotic lesions. If they are metastatic or embolic then we must assume that they migrate extensively in spite of their showing no signs of autonomous growth. On the other hand, the transformation of flat endothelial cells into higher columnar epithelial cells at several points in the same organ under the same stimulus seems to offer less difficulties of explanation.

(Dr. A. S. Warthin, Ann Arbor.) Have you ever found anything like this in the male?

(Dr. Plaut.) No, only in females where we can assume the activity of the female sex glands. Never before puberty and never more than a few years after menstruation ceased.

(Dr. Warthin.) Why then, only in one sex?

(Dr. Plaut.) I cannot answer that, but observations have led others and me to this conclusion. I have even found in older books drawings which present the same thing but without the same interpretation, of course.

(Dr. V. C. Jacobson, Albany.) I fully agree that the lesion is not a tumor. I should call it ectopic endometriosis. In other words, there are normal appearing endometrial glands plus normal stroma which at each menstrual period menstruate. I cannot conceive of strictly neoplastic tissue adhering to a normal function in such a normal way. As to why it is found in the region of the umbilicus I think the explanation in many of these cases lies in the fact that there are small umbilical hernia sacs present. Recently, I had the opportunity of studying a menstruating mass in the sac of an umbilical hernia. Endometrial tissue which had been set free in the uterine cavity had found its way into this sac. The endometrium fragments discharged at menstruation from the tubes or more often from ruptured endometrial cysts in the ovary wander around and find their way into certain places where they become anchored. The most important point in all this discussion lies in determining whether or not any of the endometrial cells cast loose at menstruation are alive. Novak and others feel that the tissue is all dead and if so Sampson's theory is wrong and Sampson's explanation just as dead. Dr. Sampson has shown (*Am. J. Path.*, March, 1927) that endometrial tissue can find its way into the uterine venous sinuses which are wide open during menstruation and a certain percentage of endometrial fragments become attached to endothelium and grow anew. They can in turn menstruate and give off more tissue which can be swept along even farther. I

fundamentalist yet, I am sure. I will say that about the same time I was interested in the heredity of cancer he treated that idea even more roughly, but even now in his own book there are suggestions of an awakening belief concerning the heredity of neoplasms. We have exactly the same situation here, in the case of the lymphoblastomatous growths, Hodgkin's disease and mycosis fungoides, that we see in the case of other neoplasms. There is a great deal of evidence as to the intrinsic nature of these conditions, particularly with reference to the family history and the family incidence, three or four cases occurring in the same family in one or different generations, and I believe the problem here is essentially that of neoplasm in general, a genetic problem. The etiology is a problem of the germ cells and the qualities contained in them. As to infections of various kinds playing any part in the etiology of this group there may be some non-specific exogenous factor that will bring out the inheritable constitutional susceptibility but these processes do not in any way conform to infections. There is no inflammation in these neoplasms except occasionally a very slight secondary one. I think the spindle cells you saw in the slides are simply flattened cells. All of the patients are dead. The course of these affections is inevitably fatal, but the fatal issue may be prolonged by irradiation. These diseases spread by infiltration; they are all essentially destructive; they have no protective function; they have all the characteristics of neoplasms and none of an infectious process. They all represent neoplastic overgrowths of the parent tissues of the blood cell forming reticulo-endothelium or endothelium and differ only in degree of differentiation.

RETICULUM. F. B. Mallory and Frederic Parker, Jr., Boston, Mass.
(See page 517.)

MENSTRUATING UMBILICAL TUMORS. Carl V. Weller, Ann Arbor, Mich.

Abstract. Two umbilical tumors presenting all the characteristics of the so-called umbilical adenomyomas have been recognized in the Department of Pathology of the University of Michigan. One of these occurred in a woman 49 years old, while the other patient was 45. In the first case there was bleeding from a small nodular umbilical tumor at each menstrual period. In the second case, only histologic evidence of menstruation was available. These tumors showed the characteristic endometrial structure in respect to both glandular elements and surrounding cellular stroma but no smooth muscle belonging to the tumor was found in either instance. The absence of such muscle has been mentioned in other reported cases and the term "adenomyoma" is, therefore, a misnomer. Greater importance is attached to these tumors from the standpoint of the evidence which they afford as to the histogenesis of this group than to their clinical aspects. Of the latter, the most important is recognition of the fact that they are neither primary melanoblastomas nor metastases of malignant intra-abdominal neoplasms, conditions with which they are usually confused clinically. As to histogenesis, the occurrence of this tumor at the umbilicus cannot be satisfactorily explained as being due to endometrial implantation or to lymphogenous or hematogenous metastasis from proliferating endometrial growths in the pelvis. It throws doubt upon the theories of Sampson in regard to the origin of this entire group and seems to find its best explanation in a theory based upon a serosal origin.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) Were these connected with any synovial cavity?

(Dr. S. B. Wolbach, Boston.) The third case was connected with the bursa beneath the patella. The other two cases developed in proximity to tendons.

(Dr. Jaffe.) Have you any idea of the frequency of these conditions?

(Dr. Wolbach.) These three cases and one other have come to our notice in about four years. One case Dr. Smith did not include in the series was sent to me from Vermont or New Hampshire. It received the same diagnosis and prognosis.

(Dr. V. C. Jacobson, Albany.) Have these any relation to the so-called xanthomatous tumors of tendon sheaths which contain many large cells filled with lipid?

(Dr. Wolbach.) I do not know. Very probably a benign form. I become more and more convinced of what I frequently say to students that the so-called classification of tumors is simply matching tumors, about like what you do when your wife sends you down town to match a fabric.

(Dr. James Ewing, New York.) I believe these tumors represent a separate group, one of the common "rare" tumors. I found one recently which began well above the patella and I have seen one which was found in a long cyst in the middle of the thigh having the same general structure. I am sure it was of the same origin. I think there is evidence of a relation between these and the xanthomatous tumors which Dr. Jacobson mentions.

MALIGNANT MEDIASTINAL DERMOID WITH MENINGEAL METASTASIS. William F. Jacobs, Buffalo, N. Y.

Abstract. The number of mediastinal dermoids and teratoma reported in the last hundred years will approximate about one hundred cases. Of these, those definitely proved malignant will be about 10 per cent.

Carcinoma predominating and sarcoma next in order, two cases are reported of chorionepithelioma and two cases mixed, carcinoma and sarcoma.

The malignant change appears to occur not only in the solid teratoid growths, but also in the simple cystic dermoids.

Case report: A white adult male, 27 years of age; occupation — locomotive fireman. Complaint, — alleged fall, some fourteen weeks prior to decease when injury was received to left shoulder and chest, causing pain and shortness of breath; during final three weeks in hospital he developed signs of meningeal irritation, positive Kernigs with cervical rigidity. The spinal fluid showed increased globulin and a cell count of 34 with distinct web formation. The diagnosis at the time of death was pulmonary tuberculosis with secondary tuberculous meningitis.

Necropsy report: The body is that of a poorly nourished adult male, five feet eight inches, with weight estimated about 130 pounds, no signs of pulmonary arthropathy.

A tumor was found in the median line over the heart. It extended upward embracing the superior vena cava. The aorta was clear. The trachea and bifurcation formed the posterior part of the tumor. It measured 14 cm. in the vertical diameter, 8 cm. lateral and 8 cm. anteroposterior. On section it was found in part cystic and part solid. The cavity contained the usual sebaceous

think this article throws much doubt on Meyer's explanation of this condition. Another thing, if the peritoneal endometriosis is due to metaplasia of the peritoneal mesothelium why is it always or so frequently found on the under and lateral surfaces of the ovary and never on the anterior surface? The explanation probably lies in the fact that the fimbriated opening of the tube is on the under and lateral surface of the ovary. That is where the endometrial tissue is generally found. I should like to ask Dr. Weller if his patient had any menstruating ovarian cysts, or masses in either ovary. That is not necessary, however. In the case I have mentioned of menstruating endometrial tissue in the sac of an umbilical hernia, we searched the ovaries carefully and found no endometrial tissue. It was another instance of peritoneal implantation without any endometrial cysts in the ovaries which are usually responsible for this ectopic endometriosis. Many things are against the metaplasia theory. In the first place, it sounds very unreasonable to me to assume that such kaleidoscopic changes can take place in peritoneal mesothelium. The presence of menstruating cysts, the facts that the abdomen and particularly the pelvis contains menstrual blood and small masses of endometrium, and that these endometrial growths are usually found on the under and lateral surface of the ovary, point to peritoneal implantation of endometrial tissue. Also, I do not believe that the peritoneum forms decidua; the decidual cells are formed from the submesothelial fibroblasts.

(Dr. Weller, closing.) In the first place in regard to whether this growth is a true neoplasm or not: If this is not a neoplasm, a myoma is not a neoplasm. The fact that this neoplasm undergoes retrogression after sexual life is over is also true of a leiomyoma. In both of the cases described the neoplasm developed without trauma and there was no history of operation or of hernia. These neoplasms grow expansively. There is no limit to their growth until the age of sexual activity is over. So far as the origin and manner of growth is concerned these facts accord with many of the criteria for a true blastoma. The suggested possibility of implantation in an umbilical hernia does not help us very much. Why do such implants never get into the foramen of Winslow where there is a peritoneal pouch provided? There are many endometrial nooks and crannies which would correspond to an umbilical hernia. I do not believe that has anything to do with it. In regard to the continuity of these glandular structures, I am of the impression that they are independent structures. I have under way now a reconstruction which should settle that question. One of these neoplasms was removed nine years ago. As far as I can trace the history that patient did not subsequently develop ovarian cysts. My history of the case is not absolutely complete. In regard to the other case no internal pelvic examination was made.

SYNOVIOMATA: A HISTOLOGIC STUDY OF THREE TUMORS OF SYNOVIAL MEMBRANE ORIGIN. Lawrence W. Smith, Boston, Mass.

Abstract. Three tumors, occurring in relation to the knee-joint, and presenting too many points in common, both clinically and histologically, to be regarded as coincidental, are presented, as a type tumor to which no definite references can be found in the literature. Their histology, as based on their embryologic origin from mesothelium, is discussed. The type cell shows multipotential differential characteristics comparable to other mesothelial tumors, such as those arising from the pleura, pericardium and peritoneum.

(commonly called dural endothelioma), the perineurial fibroblastoma (solitary neurofibroma), and the multiple neurofibroma of von Recklinghausen's disease, although the two first named tumors are fibroblastic they are easily distinguished from each other microscopically because each retains the morphologic characteristics of the specialized connective tissue from which it arises. Only in the last group is nervous tissue to be found.

In addition to the standard methods for the study of these tumors much assistance has been gained by the use of the neuroglia and neurofibril stains of Del Rio-Hortega and of Cajal. The silver carbonate method for staining connective tissue fibers has been of particular assistance.

The type cell of the meningeal fibroblastoma is the fibroblast as pointed out by Mallory. Fibroglia fibers are formed within the tumor and when slowly growing, collagen is laid down by the neoplastic cells forming broad irregular fibers. Cell structure and arrangement resemble that of arachnoid granulations.

The perineurial fibroblastoma contains a different type of collagenous fibers. In these tumors the collagen is in the form of long slender wire-like fibers which appear in parallel. Similar slender fibers can be stained in normal nerve where they are laid down in the connective tissue. They have here frequently been confused with nerve fibers. Careful study of these tumors reveals no nervous or neuroglial elements in them. These tumors are most frequently found upon the eighth nerve and the nerve roots of the spinal cord. They are rare upon peripheral nerves.

In contradistinction to the above two tumor groups the neurofibromas are multiple tumors which appear in neurofibromatosis as an expression of a system disease which often involves a large number of nerves. Nerve fibers, derived from the nerve trunk, pass through the tumor and are surrounded by a tangle of reactionary connective tissue that is in reality a magnification of the widespread pathologic alteration of the nerves which takes place in this hereditary disease. Neuroglia cells are not found in these tumors, very rarely nerve cells may be present but they then resemble ectopic cells from a nerve root ganglion. Confusion has arisen from the fact that at times within these neurofibromas perineurial fibroblastomas may appear, possibly as a result of irritation of the perineurial connective tissue. These fibroblastomas may grow so large as to displace most of the neurofibroma tissue to the periphery. Sarcomas may likewise take their point of origin in these tumors.

In the gross also these three tumor groups have characteristic features. At operation the meningeal fibroblastoma is usually dark red, attached to the dura and often degenerated at the center. The perineurial fibroblastoma is attached to a nerve, is usually brown or yellow and degeneration, if present, takes place at a number of points resulting often in multiple small cysts with shining walls. The neurofibroma, likewise attached to a nerve, is more colorless, less vascular and on cross-section the degeneration which may take place is jelly-like and translucent. Fat granular cells are absent or infrequent in neurofibromas but common in degenerative areas of perineurial fibroblastomas.

Discussion

(Dr. James Ewing, New York.) Did Dr. Penfield state exactly what was the nature of those cells?

(Dr. S. B. Wolbach, Boston.) I would like to say that I cannot be quite satisfied with Dr. Penfield's interpretations. I have studied a great number of these

material with hair. The inner wall presented a nodule from which the hairs were growing. The wall on section showed small areas of necrosis.

There was involvement of the peribronchial and paratracheal lymph nodes and the lower cervical group on the left side, also the lower pole of the left thyroid lobe.

The lungs, other than being markedly hyperemic, did not reveal any gross pathology.

The meningeal covering of the base and tips of the temporal lobes and its extensions into the Sylvian fissures were thickened, and presented numerous very fine granule-like nodules, suggesting tubercles.

The gastro-intestinal tract, liver, spleen, pancreas, kidneys, adrenals, prostate and testes were all negative.

Microscopic examination: The sections from different parts of the tumor mass revealed islands of hyaline cartilage, areas of striated muscle, smooth muscle, with a major portion made up of a dense hyalinized stroma, containing alveoli, more or less cystic with elongated columnar cells, suggesting respiratory mucous membrane. There are cystic areas lined by a very much modified stratified epithelium, showing both sudoriferous and sebaceous glands with hair follicles, and nerve fibers but no ganglionic cells. The malignant phase of the tumor is represented by dense masses of adenomatous tissue in which the cells are small, cubical, at times showing considerable variability in size and chromatin content; the arrangement in the main exhibiting an attempt to form alveoli but often conforming to no order, showing no stroma, but being in solid masses, cords and strands; nowhere does there appear any limiting membrane; similar characteristics are found in the lymph nodes and thyroid.

In the lung sections, infiltration is found along the perivascular and peribronchial lymphatics. The infiltration extends directly through the vessel wall.

The involvement of the meninges was found to be in the form of strands or cords of cells resembling those of the primary tumor without special form or arrangement. They are generally found in close relation to the meningeal vessels with some few small round cells about them in the thickened meninges.

This case ordinarily would be classed with the simple cystic mediastinal dermoids. Histologically, however, we feel that it belongs to the teratoma.

Its malignant phase is represented by an adenocarcinoma, involving lymph nodes, thyroid, lungs and secondarily the meninges.

The factor of compensation, because of accident, prompted the postmortem examination.

The clinical diagnosis was pulmonary tuberculosis with secondary tuberculous meningitis.

The gross examination, while finding the primary tumor and immediate metastatic lesions, yielded a picture in the meninges that suggested tuberculous meningitis.

(No discussion.)

THE ENCAPSULATED TUMORS OF THE NERVOUS SYSTEM. (Meningeal fibroblastomas, perineurial fibroblastomas, and neurofibromas of von Recklinghausen.) Wilder Penfield (by invitation), New York City.

Abstract. The benign tumors of the nervous system arise from a specialized investment which separates nervous tissue from the rest of the body. They may be divided on histologic grounds into three groups: the meningeal fibroblastoma

neoplastic ganglion cells then the name perineural fibroblastoma is a misnomer. I have tried to show that the solitary perineural tumor does not contain nerve fibers. The occasional tumor which contains ganglion cells belongs in the group of neurofibromas.

THE EFFECT OF HIGH VOLTAGE CATHODE RAYS ON LIVING TISSUE; AN EXPERIMENTAL STUDY. V. C. Jacobson and (by invitation) K. C. Waddell, Albany, N. Y.

Abstract. Forty white rats were exposed to the rays of the new Coolidge cathode tube, the area rayed being the unshaved abdominal skin. Voltages used were 100,000, 200,000, and 350,000 at 1 milliampere, the animals being one inch from the nickel window of the tube. The rats were killed at intervals of one minute to fourteen days following the exposure. At first the white hair in the rayed area is changed to yellow. This color gradually fades and the hair falls out. Desquamation and the formation of a superficial epidermal slough takes place, the necrosis gradually deepening until it reaches and sometimes penetrates into the muscle layer. The periphery of the burn shows practically no attempt at epithelial repair at the end of fourteen days. A dose of 200,000 volts for 60 seconds or 350,000 volts for 30 seconds, applied over an area one inch in diameter does not kill although the animal goes into general muscular spasm during the raying. The intensity of the histologic changes varies directly with the voltage. The first changes are degenerations in the epithelium of the skin and its appendages but very promptly the collagen of the corium and deeper layers is fused into a glassy mass which takes a deep black stain with hematoxylin, and orange-yellow with Mallory's aniline blue. Phagocytes attack and undermine the necrotic connective tissue thus exposing to the air the deeper tissues over which epithelium has great difficulty in growing. Elastic tissue is fused with the collagen bundles. An interesting point is that the cathode electrons or rays produce their greatest effect in a depth of only $1/10$ mm. regardless of the voltage, dead matter (tissue) stopping the electrons perhaps even more efficiently than living tissue. The deeper tissue changes such as edema, vascular congestion and leucocytosis may be secondary X-ray effects or the reaction of the body to the presence of necrotic tissue. Direct raying of intestine destroys its muscle wall in 30 seconds and of liver its parenchyma in a depth of $1/10$ mm. The profound change in the connective tissues and its effect on epithelial regeneration is being studied further.

Discussion

(Dr. A. M. Pappenheimer, New York.) What is the effect of the cathode rays on the bone marrow?

(Dr. Jacobson, closing.) I have confined the studies at present to the skin lesions, although in two rats direct raying of the exposed tibiae produced no demonstrable immediate effects. The rats, as a whole, seem to stand terrific voltage. They would go into convulsions while being rayed but come out of them without much apparent harm. A wide experimental field has been opened up by this tube. The cathode electrons produce remarkable chemical changes in substances, for instance changing castor oil into a solid, and the effect on living tissue indicates profound chemical alterations. The cathode particles themselves can apparently accomplish things not possible by the X-ray in the voltage customarily used. X-rays have never been used in voltages of 200,000.

tumors which are being more carefully studied by Dr. Percival Bailey. We have a number of meningeal tumors of rapid growth. I cannot understand if these tumors are fibroblastic in origin why none of them resembles fibromas elsewhere in the body. There are tumors of the eighth nerve which approximate in appearance the tumors of the meninges. It is sometimes impossible to tell at first glance whether we are dealing with a meningioma or a neurinoma except by consulting the sheet that comes with Dr. Cushing's specimens. I am rather reluctant to accept the conclusion that these cells in both cases are of fibroblastic origin and prefer to believe in the ganglionic crest origin as some embryologists tell us.

(Dr. H. T. Karsner, Cleveland.) Dr. Penfield's discussion of the matter is interesting and clear, but I should like to ask him about the so-called neuroma or neurinoma. I have never been satisfied that such a tumor exists without the presence of ganglion cells, which, of course, are sometimes only revealed by examination of several blocks. Does Dr. Penfield propose to eliminate the ganglioneuroma with few cells and substitute for it the perineural fibroblastoma?

(Dr. Wolbach.) I have also in the last year had an opportunity to study two neurogangliomas. A recent one was very large, extending retroperitoneally from abdomen to chest and there is no question in regard to the presence of cells of nerve origin. Here again, in this encapsulated tumor there was the histology of perineural neurofibroma together with ganglion cells and I have been of the opinion that both types of cells come from a common precursor. I hesitate to quote Dr. Bailey but I believe he holds the same idea.

(Dr. Penfield, closing.) With regard to Dr. Ewing's question about the palisading cells: They are fibroblasts. I believe the fibers that one sees between and among these cells are collagen, reticulin if you like. Those same fibers can be stained on vessels where they usually wind about more. The same fibers can also be stained elsewhere in the collagenous ribbons of which they are a differentiation or precursor. I have been unable to stain any sheath of Schwann cells upon these fibers and they have not the form of nerve fibers in the central nervous system, which are more irregular in outline. They stain with connective tissue stains.

Dr. Wolbach spoke of recurring meningeal tumors. Of course, we know the meningeal fibroblastoma can recur. Also the fact that there are other types of tumors arising from the dura, which recur, seems an objection to the term meningioma which Dr. Cushing has proposed. Meningioma would include anything, I suppose, arising from the dura or which involves the dura but which would not of necessity belong to the histologic entity meningeal fibroblastoma. I cannot answer why these tumors are not like the fibromas seen elsewhere except that the cytologic picture resembles that of arachnoid granulations from which these tumors arise. Meningeal fibroblastomas tend to invade the bone and there is a tendency for arachnoid granulations to do the same thing, which I think Dr. Wolbach has pointed out.

The perineural tumors are sometimes difficult to interpret because of a great degree of degeneration. Fibroblasts which have undergone degeneration sometimes contain nucleoli and may give a superficial resemblance to nerve cells. The ganglioneuromas are certainly at times similar in appearance to the neurofibromas, not to the perineural fibroblastomas. Both contain nerve fibers and sheaths of Schwann cells. In regard to the term neurinoma that Dr. Karsner spoke of, the name signifies that the perineural fibroblastomas contain nerve fibers which is a matter of interpretation. If they are nerve fibers and contain

by a working assumption that the observed particulate distribution of phage is only apparent and is due to its ready absorption on coarser colloidal particles of the medium. That such an assumption is more valid has been suggested by our experiments in which the number of particles endowed with specific activity of phage in a given volume of a filtrate was altered, depending on changes in the degree of dispersion of colloids in the medium. Experimental data presented in this paper strengthen this conception further by showing that the particles present in filtrates of lysed cultures of bacteria and endowed with properties of the phage are not uniform in size.

When filtrates of lysed cultures (bacteriophage) are subjected to prolonged dialysis under osmotic pressure against water, the presence of the lytic agent can be detected outside the membrane only during the first few days. The residue remaining inside the membrane contains the bulk of the original lytic agent, and yet it is no longer capable of diffusing into the outer solution.

The interruption of diffusion is shown not to be due to any alteration in the permeability of the membrane. Moreover, the residue fails to diffuse through a fresh membrane of similar permeability, while the dialyzed portion of the phage passes quantitatively through a new membrane. When ultrafiltration under pressure was substituted for dialysis, the residue on the filter could be washed repeatedly with water, or with buffer solution (pH = 7.4), without giving off into the filtrate any more active agent. However, if broth (pH = 7.4) was substituted for water, a renewed diffusion of the active agent resulted.

These results are interpreted as indicating that the colloidal particles present in the lytic filtrates, and apparently endowed with properties of bacteriophage do not represent autonomous units of the active agent, but merely serve as a vehicle on which the agent is adsorbed. They vary in size within limits wide enough to permit fractionation by means of ultrafiltration.

The fact that active principle can thus be shown to be distributed in the medium in a form of particles of different size does not necessarily deny its autonomous particulate nature, since the phage can conceivably be assumed to be a pleomorphic virus. However, the fact that the addition of broth to the non-filterable residue (presumably composed of units of the agent of larger size) allows it to pass through the membrane which held it back before the addition of broth, is contrary to such an assumption, provided, as we have shown, the permeability of the membrane was not altered by the addition of broth. Such an effect of broth might explain the occasional findings of several workers who believe they have caused spontaneous production of phage by repeated filtration of bacterial cultures, heated lytic filtrates, and even sterile broth — substrata assumed by them to be free from bacteriophage before filtration.

(No discussion.)

THE STUDY OF INTIMATE MECHANISM OF THE LYSIS OF BACTERIA BY BACTERIOPHAGE. J. Bronfenbrenner and (by invitation) R. S. Muckenfuss and D. M. Hetler, New York City.

Abstract. When susceptible bacteria are introduced into a tube of broth containing moderate concentrations of bacteriophage, after a brief period of initial lag, the bacteria begin to multiply without showing any visible effect of the presence of the phage beyond possibly a somewhat accelerated rate of multiplication. With the rapid growth of bacteria the broth becomes increasingly turbid, until suddenly and rapidly, the turbidity disappears as the result of the appar-

THE BACTERIOLOGY OF THE BLOOD OF SWINE WITH PARTICULAR REFERENCE TO THE VIRUS OF HOG CHOLERA. Paul A. Lewis, Princeton, N. J.

(Abstract not received.)

THE DEMONSTRATION OF BACTERIOPHAGE IN OLD STOCK CULTURES. Gordon M. Kline (by invitation), Albany, N. Y.

Abstract. Previous investigations of old stock cultures have demonstrated bacteriophage in only a small percentage of cases, possibly due to the methods employed. When the technic used in examining stool filtrates for bacteriophage was applied to stock cultures, lytic substance was observed in 14 of 21 cultures studied. Typical bacteriophage, as identified by transmissibility in series and formation of plaques, was obtained from 11 of the 14 lytic cultures: namely, one *B. coli*, one *B. typhosus*, two *B. dysenteriae* Shiga and seven *B. dysenteriae* Mt. Desert strains. Tubes and plates, inoculated with the bacteria only, over a period of a few months varied from complete lysis to "normal" growth. In some instances, duplicate tubes and plates, inoculated at the same time from the same bacterial suspensions, gave these opposite extremes. This spontaneous appearance and disappearance of bacteriophagic action in these cultures associates the lytic phenomenon with other spontaneous microbic variations. The seven *B. dysenteriae* Mt. Desert strains gave both large plaques (3 to 3.5 mm. diameter) and small plaques (about 1 mm. diameter), indicating the presence of at least two distinct lytic mechanisms characterized as inherited processes rather than as chance contaminations with foreign parasites. One of the Mt. Desert strains was fished from isolated colonies ten times over a period of five years, but this procedure failed to eliminate either the lytic property of the culture as a whole or either one of the lytic mechanisms active in the strain before colony isolation. Although d'Herelle states that, "the bacteria of contaminated strains are but slightly or not at all agglutinable by a specific antiserum," the agglutinability of the cultures containing bacteriophage was normal with only one exception.

(No discussion.)

THE PARTICULATE NATURE OF BACTERIOPHAGE. J. Bronfenbrenner, New York City.

Abstract. Direct, as well as indirect microscopic examination of lysed cultures of bacteria, and especially the behavior of lytic filtrates in high dilutions and their ability to cause the appearance of discrete foci of lysis in bacterial cultures on solid media have led to the generally accepted view that the active principle of transmissible lysis (bacteriophage) is present in filtrates of lysed cultures in the form of discrete particles. The uniformity of the size of these particles, as found by different investigators and by means of different methods, has in itself appeared to many to be a strong indication in favor of the conception that the particles represent the units of an autonomous organized virus, as originally suggested by d'Herelle.

However, some experiments, particularly those showing that the active principle is capable of spreading from the focus radially, independently of the multiplication of bacteria, and independently of gravity, and that the rate of its spread is conditioned by the density of the medium, seem to militate against ready acceptance of such a view. Such radial spreading could be accounted for

bacteria go on simultaneously and continuously, and at different rates in the case of different individuals. The degree of swelling of individual bacteria varies to such an extent that the results of such an analysis must, of necessity, be highly subjective, which circumstance would explain the differences of opinion expressed by different workers on this point. The actual disappearance of individual bacteria from the field of observation in the fresh preparations is so sudden and rapid that it is impossible to state with any degree of accuracy which of the bacteria have disappeared.

It occurred to us that the extent of swelling and the relation of swelling to lysis might be more definitely established if instead of attempting to follow the changes of individual bacteria, a culture were considered as a whole. If an appreciable number of cells in a culture undergoes swelling, the relative volume occupied by the solids in this culture must increase, and thus affect the viscosity of the solution. Measurements were made both by means of a capillary viscometer of Ostwald and in the torsion viscometer of du Noüy. It was found that in general the viscosity of the mixture of bacteria with a corresponding bacteriophage increases steadily up to the time when visible lysis sets in, at which time the viscosity begins to diminish, until it gradually reaches the normal level. As calculated on the basis of changes in the viscosity of the solution, at the height of swelling, bacterial bodies may occupy a volume which may be from six to twelve times as great as the original volume occupied by them. If in place of living susceptible bacteria, one employs a culture of a homologous resistant variant, or heterologous bacteria, the viscosity of the solution remains unchanged. The heated bacteriophage which is devoid of its lytic power, does not induce swelling of bacteria and does not affect the viscosity of the mixture. The fact that after lysis the viscosity reaches its original level seems to indicate that swollen bacteria must be the ones which eventually undergo lysis.

The mechanism by which swollen bacteria disintegrate, however, remains to be established. Since in the study of fresh preparations one of the difficulties in following the lysis of individual bacteria is the fact that it is not possible to predict which one of the cells will disappear, and since one's attention is divided in observing a number of cells in a field, and moreover, since the actual disappearance of the bacteria is very sudden and rapid, it has not been possible to determine what actually takes place. It occurred to us, therefore, that if the lysis were recorded cinematographically, repeated projection of the film thus obtained would permit us to single out such cells as actually underwent lysis during the period of observation, and to follow them throughout this process. This method demonstrated that bacteria apparently swell to a varying degree, and then suddenly, within less than three seconds, the outlines of the cells disappear (burst ?) and in their place there remains an amorphous residue of low refractive power. This residue in turn disappears within a short time (about one minute), leaving only a few more or less refractile granules.

This rapid melting away of the bulk of the cytoplasm, which under ordinary circumstances is supposed to consist of semisolid colloidal, highly complex material, when considered in conjunction with the changes in the density and distribution of the cytoplasm, as indicated by pictures of fresh (ultra violet light), as well as of stained swollen bacteria, suggests that it became liquefied within the cell prior to the disappearance of the cell membrane. Since such a liquefaction could be accounted for only by some enzymatic process, we were led to investigate again the question of hydrolysis occurring during lysis. It seemed to us possible that the failure of earlier attempts to detect such hydrolysis might

ent disintegration of bacteria which can no longer be demonstrated either by staining or by cultural methods.

A number of investigators have tried to elicit the mechanism responsible for this sudden disappearance. As a result, there have been recorded in the literature no less than twenty hypotheses which can be roughly separated into three main groups.

The first group of hypotheses is built up about the original hypothesis of d'Herelle, who ascribed the sudden disappearance of bacteria to bursting, due to distention incident upon the intracellular multiplication of the *Bacteriophagum intestinale*. According to this conception, the bursting of bacteria accounted, at the same time, for the disappearance of bacteria as well as for the increase in the concentration of the phage in the solution. If this hypothesis is correct, there should be no lysis without multiplication of the bacteriophage, and *vice versa* — no multiplication of bacteriophage without lysis. The opposite, however, has repeatedly been shown to occur. Under proper conditions, it is possible to obtain lysis without an increase of phage, and an increase of phage without lysis. Moreover, many investigators have failed to observe this bursting, and some state that the swelling of bacteria observed by d'Herelle is not general enough to account for the disappearance of all the bacteria, that the bulk of bacteria disappears without undergoing any visible changes in morphology, and that swollen bacteria represent the cells more or less resistant to the bacteriophage, and are dissolved very slowly or not at all.

Another group of investigators ascribes the disappearance of bacteria to autolysis. However, they have failed to show the existence of a mechanism responsible for the sudden onset and extremely rapid progress of this autolysis. Besides, thus far all chemical analyses of the cultures, after the completion of lysis, have failed to indicate any increase in protein-split products, which would be expected if bacteria had become autolysed.

The third view postulates a special type of cleavage of the bacterial cell, which breaks up without chemical disintegration of its component parts. These cellular cleavage products are endowed with an independent power of multiplication and are capable of perpetuating a similar type of cleavage in other cells of the same species. Although this view, with various minor modifications, is held by several workers, it is evident that it is very hypothetical and not amenable to experimental inquiry.

Our own attempts to follow the progress of lysis by the direct observation of fresh preparations under the microscope, as well as in stained preparations, did not bring us to any definite conclusions as to the mechanism of lysis. However, we have been able to observe more or less definite increase in the size of bacteria under the influence of the phage. The use of special staining methods showed that the cell wall of the swollen bacteria remained intact in all cases, and in no instance were we able to detect any visible indication of its rupture or bursting. On the other hand, cytoplasm showed very marked changes during swelling. It took the stain less intensely and unevenly, so that in many instances it appeared to be segmented or beaded. When such cells were photographed unstained, by means of ultra violet light illumination, the cytoplasm appeared to be of uneven density. The swollen bacteria did not stain by Congo red (vital stain), thus indicating that they probably remained alive, even when extremely distended. The extent of swelling of individual bacteria, the relative proportion of swollen bacteria, as well as the actual relation between the swelling and the lysis were very difficult to establish, because the swelling and lysis of

ADDITIONAL EXPERIMENTAL EVIDENCE OF THE INTRACELLULAR DISTRIBUTION OF TUBERCLE BACILLI. Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. Suspensions of virulent tubercle bacilli and Higgin's india ink were injected regionally in guinea pigs and rabbits and the transmission of tubercles, to other parts of the body by pigment phagocytes containing tubercle bacilli, followed. From pigmented subcutaneous tubercles in the groin, pigment cells bearing tubercle bacilli were traced to the regional lymph nodes, to the post-sternal lymphatic channels, to the anterior mediastinal lymph nodes, and to foreign body granulomas produced about inert substances in various subcutaneous areas. Pigmented tubercles produced interstitially in the ears of rabbits led to secondary pigmented tubercles of the submaxillary and peribronchial lymph nodes. The evidence obtained points to lymphatic distribution as being largely intracellular. The secondary tubercles are formed in part from wandering cells and in part from multiplication of local endothelium. Evidence is presented to show that a certain amount of intracellular distribution through blood stream is probable but is less important than in the lymphatics.

Discussion

(Dr. A. B. Wadsworth, Albany.) One question I would like to ask. I did not quite understand the technic — whether the tubercle bacilli were injected after or before the ink.

(Dr. Haythorn.) Mixed with the ink.

(Dr. Wadsworth.) I should like to ask Dr. Haythorn if he has any information as to whether or not the phagocytosis of the ink preceded or followed the phagocytosis of the tubercle bacilli in this experiment. I am much interested in the effect of the ingestion of the ink particles on the metabolic and other cellular activities and thought you might have some interesting observations in regard to the phagocytic activity of the cells that are full of ink.

(Dr. Haythorn.) The India ink cell is capable of taking tubercle bacilli.

(Dr. Wadsworth.) When well filled?

(Dr. Haythorn.) Yes.

(Dr. Wadsworth.) Was there absolutely no relationship between the phagocytosis of the tubercle bacillus and that of the ink?

(Dr. Haythorn.) I think a phagocyte is capable of taking up either and the presence of one does not interfere with the taking of the other. I know the presence of the pigment does not interfere with the taking up of the tubercle bacilli.

(Dr. H. Zinsser, Boston.) In connection with one point I have been interested — about the idea that the first contact of the tubercle bacillus was really with the phagocytes. Polymorphonuclears might carry it.

(Dr. Haythorn.) Dr. Gardner has done more work on that than I. As he is present, perhaps he will answer.

(Dr. L. U. Gardner, Saranac Lake, N. Y.) I have made a particular study of this problem in the peritoneal cavity of guinea pigs. Bacilli were found within eosinophils during the first half hour after inoculation. From one to four hours many were phagocytized by neutrophilic polynuclears and some by mononuclear leucocytes. By twenty-four hours practically all of the bacilli were found within mononuclear cells. I do not think phagocytosis by eosinophils is general as I have found it in no other animal except the guinea pig.

conceivably have been due to the fact that the material subjected to analysis consisted of culture medium too rich in various products of hydrolysis to permit the detection of a possible small increase due to the lysis of bacteria. We therefore grew bacteria in the presence of the phage on synthetic medium devoid of all protein, and under this condition we have been able to detect unmistakable evidence of an increase in amino nitrogen as a result of the lysis of bacteria.

(No discussion.)

TULAREMIA, HISTOPATHOLOGY OF THE LESIONS IN MAN. Edward Francis (by invitation) and G. R. Callender, Washington, D. C.

Abstract. The lesions of tularemia in man may be divided into the local lesions at the primary point of inoculation, the secondary adenopathy subsequent to a primary lesion or without any evident point of entrance and the generalized lesions throughout the parenchymatous organs. The histopathology of these lesions was presented and illustrated. The early lesions are focal necroses, such reaction being followed relatively rapidly by endothelial proliferation and fibroblastic increase. The greater the duration of the disease the more marked is the fibroblastic response of the tissue to the infection. The similarity to certain types of focal lesions of tuberculosis was stressed and differential points considered. The histologic appearances alone are not considered diagnostic and should be differentiated from tuberculosis by special stains and inoculations and verified for tularemia by the agglutination reaction. Ten cases of the disease in man formed the basis of the study, three of which were fatal.

Discussion

(Dr. M. W. Lyon, Jr., South Bend.) I rise to a point in mammalogy. Dr. Callender says there are not as many rabbits in this part of the country as where he comes from. I am willing to concede there are not as many seen in public markets as one sees in Washington, but rabbits of the genus *Sylvilagus* are very common animals in the northern tier of the middle central states. The form occurring there is *S. floridanus mearnsi*, while the form commonly found around Washington and Baltimore is *S. floridanus mallurus*. This difference in races of rabbits may have some bearing on the distribution of tularemia, though the climatic factor is probably more important.

(Dr. H. Zinsser, Boston.) We have recently run into a case of abortus infection, an experimental infection in a young physician. In considering the diagnosis the question arose as to what extent agglutination overlapped in the two diseases. Can you give us a little information as to the difference in the serologic diagnosis and the similarity of the two infections in man?

(Dr. Callender, closing.) With reference to Dr. Lyon's remarks — I do not know that all rabbits are susceptible nor do I know of any work that has been done along this line. All rodents so far inoculated are extremely susceptible.

I am not sufficiently familiar with infection with *B. abortus* to discuss the similarities or dissimilarities between the two diseases.

With reference to the diagnosis, patients infected with *B. abortus*, *B. melitensis*, or *B. tularensis* show agglutination of all three organisms, and the titer may not be significantly higher in the disease which affects the patient. Absorption tests differentiate easily and clearly so that there is no doubt in the mind of the bacteriologist.

Discussion

(Dr. L. W. Famulener, New York.) I would like to ask what culture medium and the age of the culture medium.

(Dr. Medlar.) I was not particularly interested in cultivating the organism but I found Sabouraud's medium best. I also found that factors such as moisture apparently govern the production of the aerial hyphae and fruiting bodies.

(Dr. Hans Zinsser, Boston.) You did not mean to say, Dr. Medlar, that you thought there was any danger of a pathologist confusing the two conditions.

(Dr. Medlar.) I do not see how you can make the diagnosis unless blastomycetes were present.

(Dr. Zinsser.) It seemed to me in our own studies that there was considerable difference — the diffuseness and the characteristics of the giant cells. I wondered if you thought that a competent pathologist might make a mistake.

(Dr. Medlar, closing.) In fact, most cases of blastomycosis resemble more an acute type of infection. Our first case was one in which the individual had a generalized blastomycosis which had lasted from a few months to a year. Our second case was one in which we had no idea how long the infection had existed. It shows a much more typical tuberculous picture histologically than the acute case. Without the presence of blastomycetes in this last case I could not tell the difference histologically between blastomycosis and tuberculosis.

COCCIDIOIDAL MENINGITIS: G. Y. Rusk and (by invitation) L. W. Buck, San Francisco, Cal.

Abstract. A report of four cases of basilar meningitis due to the above organism. History suggestive of pulmonary infection in two cases. In these, however, all pulmonary symptoms subsided and in one case which came to complete necropsy no infection was found outside of the central nervous system, except one possible collapsed organism in a section of lung. The cases ran a course of chronic hydrocephalus or other form of intercranial pressure, at times suggesting tuberculous meningitis, brain tumor, or hydrocephalus. Operative procedures were instituted in all cases. Demonstration of the material showing the characteristic organism.

(*No discussion.*)

SOME POINTS ON THE MECHANISM OF FILTRATION BY THE SPLEEN. W. L. Robinson, Toronto, Can.

Abstract. The histologic structure of the spleen, with its arterio-capillary system opening out into the pulp sinuses to bring the blood into direct contact with the vast network of reticulo-endothelial cells comprising the pulp, would seem to have as one of its purposes that of filtration.

Filtration experiments were done by intravenous injections and perfusions of fresh isolated spleens. As substances for filtration, India ink, acid and basic dyes, colloidal solutions of copper, platinum and silver, bacterial suspensions and red cells stained with eosin, were tried.

The capillary walls as they pass through the ellipsoids and continue on to open out into the pulp are permeable for fluids, colloidal solutions and suspensions of fine particulate matter. The red cells, as has been suggested before, apparently pass on through the end capillaries into the Ampulla of Thoma and the pulp sinuses. This serves as a mechanism for separating the plasma with its foreign material from the red cells.

In highly allergic human beings acute inflammatory exudates are often seen which are composed largely of polynuclear leucocytes. Tubercle bacilli are sometimes very abundant within these cells. As examples may be mentioned certain phases of tuberculous meningitis, and acute tuberculous bronchopneumonia.

(Dr. Haythorn.) During the early stages the tubercle bacilli were only found in the large mononuclears but there is a stage in which the polymorphonuclears were very active during the caseation of the tubercle. I have not found any polymorphonuclear leucocytes carrying tubercle bacilli but I do not consider my experiments conclusive on that point.

(Dr. H. H. Permar, Pittsburgh.) The reason that Dr. Gardner did not hear the question at first was because we were discussing the reaction about the lycopodium spores. Was the reason the tubercles did not form around the lycopodium spores because the cells which had phagocytized tubercle bacilli did not move so rapidly as those which contained only carbon pigment?

(Dr. Haythorn, closing.) You put carbon pigment and tubercle bacilli into the groin and the large mononuclears take up a great deal of the pigment rapidly but there is still pigment which is not taken up immediately, and that is left free when the tubercle begins to form. The pigment cells included in the formation of tubercle are held together in the little granulomatous mass during development. There are usually some pigment cells in the surrounding tissue not included in the tubercle. When I waited for from fourteen to seventeen days and put in lycopodium I got tubercles formed by pigmented cells containing tubercle bacilli which I believe were freed by the breaking down of the nodules. At times I put in a little lycopodium. If I was too early I got only the pigment cells not at that time included in the formation of the tubercle, but after caseation I got tubercles formed by the pigment cells presumably freed by breaking down the tuberculous nodule.

PULMONARY BLASTOMYCOSIS; ITS SIMILARITY TO TUBERCULOSIS. REPORT OF TWO CASES. E. M. Medlar, Madison, Wis.

Abstract. The two cases upon which this report is based are cases of primary pulmonary blastomycosis. Skin lesions did not precede the pulmonary manifestations. Case I was a young male aged 17 years. He died of the infection about eleven months after the onset of the disease. He had generalized infection with many joints and bones involved. This case represents the acute type of blastomycotic infection.

Case II was a male age 65 who died from cardiac decompensation. He gave no history of pulmonary or skin lesions suggestive of either blastomycosis or tuberculosis. Necropsy showed pulmonary lesions indistinguishable from old tuberculous lesions.

On microscopic study no essential difference in the histopathologic lesions could be determined if the acute phase of the tuberculous lesion was compared with the acute blastomycotic lesion or if the chronic phases of the two diseases were compared. In other words, when the virulence of the infectious agent is considered the lesions produced in these two diseases are indistinguishable. Giant cell formation appears to be brought about in the same way. Caseation appears to be produced in the same manner.

From this study it appears that the reaction to the tubercle bacillus is not specific. A similar pathologic reaction occurs in blastomycosis.

Discussion

(Dr. J. Ewing, New York.) The splenic lesion which Dr. Klotz has so minutely described reminds me of the splenic lesions one sees in cases of intestinal intoxication. I am surprised too that one should discuss yellow fever without reference to leptospira.

(Dr. A. B. Wadsworth, Albany.) I would like to ask about hemorrhagic lesions.

(Dr. S. B. Wolbach, Boston.) I should like to know whether he succeeded in transmitting this form to guinea pigs.

(Dr. H. Zinsser, Boston.) Does anyone else wish to ask another question or the same question in another form? I will ask Dr. Klotz if he wishes to answer.

(Dr. Klotz.) The work which was carried out during the past year by the Yellow Fever Commission is not completed, and further studies are to be carried on during this and succeeding years. Hence the findings which have been reported to the present can only be considered in conjunction with those which are to follow. In answer to Dr. Wadsworth's question as to the presence of hemorrhagic lesions, it was noted that these were present in petechial or small blotchy areas in the skin, lungs, stomach, intestines and serosal surfaces, but their distribution and extent were very variable. We were unable to demonstrate leptospira in the tissues obtained at necropsy.

(Dr. Wolbach.) Was transmission to guinea pigs absolutely negative?

(Dr. Klotz, closing.) The laboratory work in bacteriology and immunology was carried out by Dr. Henry Muller who was in charge of the laboratories of the Commission, and Dr. Kligler. Guinea pigs were found not to be susceptible to infection by inoculation of blood from cases of West African Yellow Fever, and to the present no leptospira was isolated on culture media. The British officers both in Nigeria and the Gold Coast were most cordial in their coöperation, and gave every facility to carry on the work.

HISTOLOGICAL AND CHEMICAL OBSERVATIONS UPON THE ORIGIN OF THE POLYCYSTIC LIVER. Ernest Scott (by invitation), Columbus, O.

Abstract. From a review of previously reported cases and three cases observed in this laboratory, histologic investigation shows that polycystic liver is a condition associated with an increase in the periportal bile ducts. Previous investigators have attributed this increase to inflammation, neoplastic growth and congenital anomaly. From investigations of the three cases in this laboratory it is our opinion that the fundamental lesion is an inflammation in the nature of a periportal cirrhosis. Since all three of the cases reported were in adults, it is impossible to say if this statement holds for the embryonic type of polycystic liver. Physical chemical determinations on the cyst fluid reveals the absence of bile and proves beyond question that the cyst fluid is the product of selective secretory activity.

(No discussion.)

THE ETIOLOGIC STUDY OF THE PATHOLOGY OF EIGHTEEN CONGENITAL POLYCYSTIC KIDNEYS. James E. Davis, Detroit, Mich.

Abstract. The material studied was from subjects varying from fetal life to 65 years, one at five months, one at seven and one-half months, four at term, one

Small quantities of India ink solution injected intravenously were practically all filtered out by the ellipsoids. Beyond the ellipsoids the first trace of India ink adherent to the pulp cells was found about the malpighian follicles.

Freshly isolated spleens perfused first with a 2 per cent solution of potassium cyanide filtered the India ink just as readily as the living spleen in intravenous injections. The process of filtration does not appear to be a vital one.

The pulp cells even when the pulp spaces were expanded to their capacity by the perfusing fluid, quite readily filtered the minute particles of India ink. The process of filtration is not, essentially, mechanical in nature.

In all cases the fine particles of foreign material were found firmly adherent to the filamentous processes of the pulp or ellipsoid cells and could not be dislodged by the blood plasma or the perfusing fluids. That the process might be one of adsorption is suggested by the fact that while India ink and platinum and silver colloidal solutions all carrying negative electrical charges were readily filtered out both in intravenous injections and perfusions of the isolated spleen, the copper colloidal solution which carries a positive charge apparently passed through the splenic pulp without filtration.

(No discussion.)

THE SPLEEN IN WEST AFRICAN YELLOW FEVER. Oskar Klotz and (by invitation) Winifred Simpson, Toronto, Can.

Abstract. Very little attention has in the past been given to the changes which occur in the spleen in yellow fever. The studies in the pathology of the disease have centered upon the lesions of degeneration which are particularly prominent in the liver and kidney, but which also affect the heart. One of the prominent characteristics in yellow fever is the lack of inflammatory reactions in any of the organs. The disease is one in which a toxemia severely injures and induces necroses which are commonly preceded by a fatty degeneration.

In a study of the spleen it was found that there was a fairly constant change to be observed in the fatal cases. The malpighian bodies show a marked disappearance of their lymphoid elements, while the endothelial cells within them appear more numerous. In the early stages of the reaction the germinal centers of the malpighian bodies may show some hyperplasia of their endothelial cells which is soon followed by a hyaline degeneration and necrosis. The lymphoid follicles tend to disappear and in their peripheral portions the endothelial cells in the sinusoids become free. These cells tend to enlarge and form irregular multinucleate masses. During the degeneration of these endothelial cells the chromatin of the nuclei becomes scattered through the cytoplasm and not infrequently resembles protozoan parasites. In the pulp spaces at some distance from the lymphoid follicles the tissues are injured but no definite hyperplasia of the cells of the reticulo-endothelial system can be made out. In those cases where the spleen has been severely affected, débris arising from the dead endothelial cells can be discerned in the sinusoids.

The reaction, although not a specific one for yellow fever, assists in making a differential diagnosis from other diseases which in the tropics may simulate it. It is distinctive from that which arises in relapsing fever and infectious jaundice.

(Dr. Maude L. Menton, Pittsburgh.) Any special stains?

(Dr. Robertson.) They did not work.

(Dr. H. T. Karsner, Cleveland.) This report is of great significance and interest. Goldblatt (*J. Cancer Research*, 1921, vi, 277) reported a case of benign adenoma of the islet tissue but found few similar cases in the literature. The diagnosis, of necessity, rested upon a presumptive identification of the type cell. Robertson has the additional evidence of a special functional capacity of the tumor to aid in its identification. This case brings to mind the old question of preservation of function of cells after they have reverted to a highly active rate of multiplication.

RIGHT-SIDED AORTA (PERSISTENCE OF THE RIGHT AORTIC ARCH). Aaron Arkin, Chicago, Ill.

(*Abstract not received.*)

COARCTATION OF AORTA (ADULT TYPE) WITH COMPLETE OBLITERATION OF DESCENDING ARCH. Maude E. Abbott, Montreal, Can.

Abstract. A case is reported of complete obliteration of the descending arch at the point of insertion of ligamentum arteriosum with persistence of the fetal isthmus and anomalous (persistent left fifth?) arch. Bicuspid aortic valve, aortic insufficiency and subaortic stenosis in a lad of 14.

The collateral circulation was extensively developed and the ascending aorta had undergone aneurysmal dilatation with dissection and impending rupture of the right anterior wall. The diagnosis was made during life on the basis of the destructive symptomatology, which is discussed in the light of the necropsy findings. The literature on rupture of the aorta in coarctation of the adult type is briefly reviewed.

(*No discussion.*)

THREE SPECIMENS OF HEARTS SHOWING CONGENITAL LESIONS. B. L. Crawford and (by invitation) Edward Weiss, Philadelphia, Pa.

(*Abstract not received.*)

REPORT OF SEVENTY-THREE CASES OF PULMONARY EMBOLISM. J. S. McCartney (by invitation), Minneapolis, Minn.

Abstract. Seventy-three cases of pulmonary embolism were found among the 9,275 necropsies on record in the Department of Pathology of the University of Minnesota. They were divided into four groups (1, *a*) post-traumatic, 15 cases; (1, *b*) post-traumatic with operation in consequence of injury, 8 cases; (2) post-operative, 31 cases; (3) postpartum, 3 cases; (4) miscellaneous, 16 cases. There were 40 males and 33 females. The age varied from 19 to 83 years. When considered in relation to the number of necropsies done in each decade, this series shows practically the same incidence of embolism in all decades after the first. Age is probably not as important a factor as it is usually thought to be.

In the postoperative group, wound infection was present in 17 cases. In 27 of the 31 postoperative cases embolism occurred during the first two weeks.

Apparently the occurrence of thrombosis and pulmonary embolism after injury is not generally well known. One hundred and nineteen cases of post-traumatic thrombosis and embolism were collected from the literature. These

a day old, one adult 22 years of age, another 32, six between 40 and 50, three at 45, 55 and 65 years of age, respectively; two were from the negro race.

There were other multiple deformations in 50 per cent of the cases.

There was definite history of inheritance in 55 per cent of the cases. In two generations there were ten instances of congenital polycystic kidneys and three specimens were obtained from one family. Three of the cases were operated upon and only one has survived operation for a period of two and one-half years after her nephrectomy.

The material showed in all instances developmental defects in both cortex and medulla, the earliest formation occurring quite uniformly in the subcortical zones in kidneys, and in certain instances in liver and spleen. In the kidneys, delayed and defective development occurred in all parts of the majority of the nephrons. Delayed development of glomeruli, convoluted tubules and collecting tubules was evident in all cases, but in addition this deficient development was always found in areas of embryonic or mesenchymal stroma.

In all instances the details of differentiation phenomena were observed from columns of unassembled mononuclear cells in mesenchymal stroma to partial assembling of these cells as straight segments with here and there partial development of lumina to advanced cystic degeneration in such areas, whether found in the straight tubules, convoluted tubules or Bowman's capsules.

Conclusion. Structural etiologic defects preceding bilateral congenital polycystic kidney degeneration are due to delayed development of entire nephronic units and their surrounding stroma.

(*No discussion.*)

A CASE OF HYPOGLYCEMIA, PROBABLY PRODUCED BY A CARCINOMA OF THE ISLANDS OF LANGERHANS. H. E. Robertson, Rochester, Minn.

Abstract. This is a report of the pathologic conditions found in a case of hyperinsulinism and hypoglycemia which were studied and are to be published by R. M. Wilder, F. N. Allan and H. E. Robertson.

The patient, a physician aged 40, had had pains in the upper abdomen for a long period and more recently attacks of jaundice and weakness which could be relieved or prevented by the administration of sugar, the requirements increasing until just before death when not less than 25 gm. of glucose was necessary each hour. At postmortem examination a degenerated malignant tumor was found in the tail of the pancreas with metastasis to the adjacent lymph nodes and liver. The liver and kidneys were enlarged. Microscopically the tumor cells resembled those of the islands of Langerhans in structure and arrangement. The tumor tissue in the liver was found to contain insulin. The liver showed rich deposits of glycogen. The tumor was adjudged to be a functioning carcinoma arising from the islands of Langerhans.

Discussion

(Dr. James Ewing, New York.) It looks to me like parenchyma cells, but I do not understand why the glycogen was not burned as well.

(Dr. Robertson.) I believe that the glycogen was mobilized in the liver due to the huge amounts of sugar administered. Just how that happens I am not able to say. The facts are that the patient was suffering from hypoglycemia and hyperinsulinemia and it would appear that this tumor contains an insulin-like substance.

end of three months you usually should have some organization of the thrombus in the vessels. I have made no microscopic examination of any of these specimens. This is simply a statistical study of the cases, no microscopic work being done. Certainly, in our service we see every now and again cases where the clinical history indicates pulmonary embolism but we do not always find embolism. I think that is the experience of everyone. I am sure I do not know how to account for it. None of these cases followed a manipulation such as Dr. Jaffe mentioned in orthopedic hospitals. I have seen such cases in the literature and probably the explanation is the same as in the others, namely that the vein is traumatized and the intima torn.

PULMONARY ARTERIOSCLEROSIS ASSOCIATED WITH PRIMARY CARCINOMA OF THE LUNG. William Boyd, Winnipeg, Can.

Abstract. The etiology of Ayerza's disease is still obscure. Some cases appear to be due to syphilis. In others the pulmonary arteriosclerosis is associated with conditions in which there is long-continued increase of pressure in the pulmonary circulation, such as mitral stenosis, emphysema and chronic pulmonary tuberculosis. In the case described in this paper none of these factors was present, but the pulmonary arteriosclerosis was associated with carcinomatosis of both lungs. The relationship between the two conditions is discussed.

Discussion

(Dr. A. S. Giordano, South Bend.) I wonder whether or not the microscopic sections of the prostate and of the rectum were carefully searched. The gross picture of the lung is a little bit unusual for a primary carcinoma of the lung. I was wondering if there was a possibility of primary carcinoma elsewhere. I have more than once been embarrassed to find what I considered primary malignancy in the lung, not to be primary in the lung at all.

(Dr. Alfred Plaut, New York.) Would Dr. Boyd like to give an opinion as what he thinks of the causal relationship or of the coincidence?

THE INVOLVEMENT OF THE AORTIC VALVE IN SYPHILITIC AORTITIS. Otto Saphir and (by invitation) R. W. Scott, Cleveland, O.

Abstract. The most constant histologic findings of the aorta in the region of the sinus of Valsalva are endarteritis obliterans of the vasa vasorum and perivascular infiltration of lymphocytes in the adventitia. These vessel changes lead to necrosis of the media and mucoid degeneration of the inner portion of the media and intima. The degenerated areas become organized as indicated by new formation of vessels and the presence of endothelial cells, fibroblasts and young connective tissue. The perivascular infiltration about the newly formed vessels and the presence of lymphocytes throughout show that the process does not undergo healing, but proceeds continually as a chronic inflammation. Older cases show hyalinization of these areas. The constant presence of the newly formed vessels prevents ulceration. The lateral portion of the aortic valve leaflets which according to Bayne-Jones is supplied by the vasa vasorum from the aorta shows similar degenerative changes as the intima and media of the aorta. Organization of both sides leads to adhesions between the lateral portion of the valve and the aortic wall of the sinus of Valsalva leading to the gross picture of a separation of the commissure.

(Discussed with next paper.)

together with the twenty-three reported here make a total of 142. From these 142 cases certain general conclusions are drawn. (1) Post-traumatic thrombosis and embolism are not at all rare; (2) a simple fracture was present in a majority of instances, but only rarely a compound fracture; (3) in a number of instances there was only minor bruising of the soft tissues without fracture of a bone; (4) the thrombus always developed at the site of the injury; (5) the interval between trauma and pulmonary embolism is longer than that between operation and embolism; (6) the age distribution seems to be about the same as that of other forms of pulmonary embolism; (7) injuries resulting in embolism usually involve the lower extremities.

In postoperative thrombosis and embolism the primary site of the thrombosis may lie within the operative field or be distantly removed from it. The iliac, femoral and pelvic veins are the most common sites of the primary thrombosis. The veins of the left side are most often involved. Postoperative pulmonary embolism usually follows operations below the level of the diaphragm, and only rarely those above the diaphragm, and is most common following operations on the prostate, intestine and biliary tracts.

Thrombosis and embolism develop in a great variety of medical conditions, and the thromboses are usually situated in the veins of the lower extremities, as in postoperative cases, often independent of the major anatomic lesions.

Discussion

(Dr. M. W. Lyon, Jr., South Bend.) I would like to ask if figures for simple and compound fractures have been corrected for the frequency to the one and the comparative infrequency of the other.

(Dr. McCartney.) I took all the cases here in one group without making correction for the difference in incidence of the two types. I did so for the reason that in the literature it is frequently stated that embolism is more likely to follow a compound rather than a simple fracture.

(Dr. W. W. G. MacLachlan, Pittsburgh.) In the cases which we see in Pittsburgh in the coal miners, where compound fractures are very common and often multiple, it is very rare in these severe fractures to meet with an embolism at necropsy. The few that we have seen have all occurred from minor fractures or fractures not in good position, and operated on. In the very severe injuries, and the coal miners get terrific injuries, it is rare to find embolism.

(Dr. William Boyd, Winnipeg, Can.) Is there any explanation to offer about what exactly was happening in the case where the embolism did not occur for ninety days? Has anybody come across those postoperative cases where the patient dies with all the symptoms of pulmonary embolism but necropsy fails to reveal any embolus even if done with the greatest care. We have had two or three cases of that sort.

(Dr. H. L. Jaffe, New York.) I wonder whether any evidence of embolism has been noted in those cases in which there has been no injury but just manipulation of bone or joint in a closed operation. One sees suggestive cases in orthopedic hospitals following such procedures. The patient leaves the operating room and within a few hours after going to the ward dies with symptoms like embolism and careful necropsy fails to disclose the cause of death.

(Dr. McCartney, closing.) I have not any explanation to offer at all for the long interval between the injury and the embolism. It would seem that by the

clinical picture of aortic insufficiency although at necropsy the valve is little distorted. The ring itself is dilated perhaps but has perfectly normal appearing aortic leaflets. I wonder if Dr. Clawson has observed such cases, also whether or not he has seen a syphilitic distorted aortic leaflet in which the commissure was not widened.

(Dr. Saphir.) I wonder, too, if you have found any separation of the commissure of the two leaflets without hyaline plaques above the leaflets of the aorta.

(Dr. Clawson, closing.) From the microscopic condition of the valve I regard the pathogenesis of the thickening as inflammatory in character. The amount of proliferation which I showed you on the slide had not been produced from just some other injury. This inflammatory condition starts from the aorta and is known to extend beneath the endothelium of the valve on both sides of the valve but not from the sinus of Valsalva. There are many cases of aneurism without valvular involvement but as a rule the hearts are small and the valves in many of them are not involved and there is nothing to stimulate hypertrophy. In regard to the stretching of the orifice in three of my cases the cardiac failure cannot be accounted for on a vascular basis so we figured that in these cases stretching of the aorta must have had something to do with it. Unfortunately, the width of the aorta was not measured at the time of necropsy and I thought the measurement of the formalin specimen would not be correct. I have measured the aortic ring since in typical cases of aortic insufficiency with injury to the valve and have found that the diameter of the aortic orifice was normal. I have not worked up the microscopic structure of the aorta but in regard to the hyalinized plaques above the attachment of the valve you always have a proliferation which pushes the attachment of the aortic cusps apart somewhat. At first, it may not be hyalinized but may become so later.

PERIARTERITIS OBSOLETA NODOSA. A STUDY OF THE HISTOLOGICALLY HEALED END-STAGE. Aaron Arkin, Chicago, Ill.

(Abstract not received.)

A BLOODLESS METHOD FOR TAKING REPEATED BLOOD PRESSURE READINGS IN LABORATORY ANIMALS. Leone McGregor (by invitation), Minneapolis, Minn.

Abstract. Investigators attempting to produce chronic arterial hypertension experimentally find it necessary to take a long series of blood pressure readings on each animal. With the method of a cannula in an artery only a few readings are possible.

My method is a combination of the procedures used by Fahr and Allen. The rabbit is tied in the dorsal position. The phonendoscope is applied over the termination of the abdominal aorta, where it may be held in place by tapes. The cuff is wound around the lower abdomen just above the iliac crests, so that the phonendoscope is just inside the lower border. As Allen has pointed out, the sounds are more clearly heard when the phonendoscope is inside the cuff than when it is distal to the cuff as in clinical procedures.

The readings are taken in the usual manner. The cuff is inflated until the aortic pulse disappears. Then the mercury is allowed to fall. The first sound which comes through is taken as the systolic pressure. With a further fall in the

THE HEART IN SYPHILITIC AORTITIS. B. J. Clawson, Minneapolis, Minn.

Abstract. One hundred and twenty-six hearts associated with syphilitic aortitis are studied to observe the anatomic changes in the valves, coronary arteries, myocardium and pericardium, and to note the immediate relation of these changes to the cause of death.

The 126 cases on the basis of their clinical courses and the pathologic findings at necropsy are classified as follows:

1. Aortic insufficiency, 48; 36.5 per cent.
2. Sudden death from closure of coronary orifices, 25; 19.9 per cent.
3. Rupture of aortic aneurism, 35; 27.7 per cent.
4. Gummata of myocardium, 3; 2.4 per cent.
5. Miscellaneous (death from other causes), 17; 13.5 per cent.

Syphilitic valvulitis (a cord-like thickening of the free margins of the cusps) and a separation of the aortic cusps at their attachments to the aorta are commonly associated with syphilitic aortitis. The gross injury to the valve regularly produces an insufficiency but never a stenosis. Narrowing of the coronary orifices to the extent of producing death is common.

Aside from the cases with rupture of an aortic aneurism and the few cases with gummata of the myocardium, death is practically always accounted for by the aortic valvular injury with insufficiency or the narrowing of the orifices of the coronary arteries. Sudden death with syphilitic aortitis is rarely due to a myocardial inflammatory condition.

Discussion

(Dr. Otto Saphir, Cleveland.) We have noted in our cases thickening of the midportion of leaflets of the aortic valve. We do not find there any changes or any signs characteristic of syphilis. We think that the cord-like thickening of the valves is just due to chronic inflammation caused by the regurgitation of blood after the insufficiency of the valve has been established.

(Dr. W. W. G. MacLachlan, Pittsburgh.) We have been interested in the relation of the aortic valves to aortitis, chiefly through some observations in cases of aneurysm where we found, clinically, no evidence of aortic insufficiency and in others at necropsy the presence of aneurysm with normal aortic cusps. We feel that there are a certain number of aortitis cases where the process does not go much below a point about one inch above the aortic ring. We agree with the findings of Dr. Scott and Dr. Clawson that lues produces a thickening of the aortic cusps, but some cases, even advanced to degrees like aneurysm, do not appear to show this change. After the age of 50 I think one must consider the change that may occur at that time of life due to sclerosis. We have been impressed on the clinical side by the fact that students invariably interpret an aortic regurgitation in an adult as being due to lues and to aortic valve disease, and do not seem to consider that an aortitis may be present with no valvular involvement.

(Dr. R. W. Scott, Cleveland.) We are very pleased to hear that Dr. Clawson's work, which agrees in every detail with our own observations of the last seven or eight years, indicates that whether a patient having syphilis of the aorta gets signs of heart disease or not depends not upon what happens in the myocardium but whether or not the process attacks the roots of the aorta and the valves. In regard to the distortion of the architecture of the leaflets one sees an occasional

Just one vein was used all the time. That produces no change in the blood chemistry. Of course you can take one kidney out and that has no effect. As to what the blood pressure is in the rabbit in the aorta: I heard a paper in the Physiology section yesterday in which blood pressures of 130 were reported in the aorta of the rat. In the rabbit by our method they were 110 to 140 as a general rule. This is a little higher than the pressure obtained with a cannula in the aorta. In discussing this with my physiological friends they thought it a little more accurate than the cannula inasmuch as we did not have to deal with the inertia of the column of mercury. Where the rabbit had a lot of arteriosclerosis in the aorta, and in many cases there was a great deal, there was no sclerosis in the small arterioles. With cholesterol feeding there is marked atheroma but that never goes beyond the large arteries.

ANALYSIS OF FOUR HUNDRED CASES OF PRIMARY HYPERTENSION. E. T. Bell, Minneapolis, Minn.

Abstract. Four hundred and twenty cases of primary hypertension that came to necropsy have been studied. Primary hypertension includes all cases with persistent or intermittent high blood pressure of unknown cause; also all cases with a heart weight of 500 gm. or more in males and 450 gm. or more in females, all known causes of cardiac hypertrophy having been excluded. In accordance with the manner of death the group may be subdivided into myocardial insufficiency, coronary disease, apoplexy, renal insufficiency and miscellaneous.

Ninety per cent of the patients were over 40 years old, and 74 per cent were over 50 years old at the time of death. In our necropsies 15 per cent of persons over 50 years of age had hypertension. The renal group are somewhat younger on the average than the rest. The proportion of males to females is about 1.4 to 1. The blood pressure tends to be highest in the group with renal insufficiency. Evidence is offered to show that all cases of idiopathic hypertrophy and dilatation of the heart with congestive heart failure are instances of primary hypertension. Gross sclerosis of the coronary arteries is more frequent and more severe in hypertensive than in non-hypertensive heart disease of corresponding age. There is some evidence that a large percentage of cases of clinical coronary disease are closely related to hypertension. It is highly probable that all cases of apoplexy on an arteriosclerotic basis belong in the hypertension group. The arteries in the parenchyma of the kidneys show some sclerosis in 97.6 per cent of cases of hypertension (severe in 61.7 per cent, slight to moderate in 35.8 per cent). The renal arterioles show sclerosis of varying degree in 88.1 per cent of all cases of hypertension. The coronary and miscellaneous groups show the lowest incidence of arteriolar sclerosis.

Discussion

(Dr. O. Saphir, Cleveland.) I would like to ask Dr. Bell if he found any fat in the arterioles of the kidneys.

(Dr. A. M. Pappenheimer, New York.) I would like to know what his ideas are on the relation of arteriosclerosis of the arterioles to arteriosclerosis in general. Is it a separate manifestation of the same thing or different altogether?

(Dr. H. T. Marshall, University, Va.) I would like to ask a question in regard to the fourteen cases in which there was no increase in the weight of the heart above the limit selected. The majority of our cases at least are accustomed to develop muscular size in proportion to the exercise but even some of the best

mercury the sound becomes softer. This is taken as the diastolic pressure. The aortic sounds are sharp and distinct and the pressure can be read as accurately as in man. The normal aortic systolic pressure in rabbits varies from 115 to 140 mm. Hg. Adrenalin shows the same sharp rise as occurs when the pressure is measured with a cannula in the carotid. The blood pressure can be read as accurately in monkeys as in rabbits.

(*No discussion.*)

A METHOD OF PRODUCING EXPERIMENTAL CHRONIC HYPERTENSION IN THE RABBIT. A. H. Pederson (by invitation), Minneapolis, Minn.

Abstract. The kidney is approached by the posterior route. A longitudinal incision is made about one and one-half inches to the left of the mid-dorsal line, and the lumbodorsal fascia is exposed. Following the line of cleavage and separating the muscles in the direction of their fibers, the perirenal space is entered. With a little gentle pressure on the abdomen, the kidney is pushed out. The fatty capsule is stripped off and the renal vein and artery are identified and separated. An aluminum wire band is put around the renal vein, and sufficiently constricted so that the kidney becomes tense and purplish. To prevent the development of collateral circulation, the whole kidney is placed in a loose pouch of fixed animal membrane. The opening is surrounded by a purse string suture drawn about the renal pelvis and vessels. The kidney is then replaced, and the wound sutured in layers.

In the five rabbits that have survived the operation for two weeks or more, there has been a definite elevation of the aortic blood pressure, from 110 to 130 (normal) to 180 mm. Hg. or higher. The highest pressure recorded was 270 mm. Hg. The blood pressures were read by Dr. Leone McGregor by the method she has just demonstrated to you. One rabbit which has survived the operation for fifty days has a blood pressure of 180 mm. Hg. It is suggested that the hypertension is of reflex origin, due to increased resistance in the circulation through the obstructed kidney.

Discussion

(Dr. R. W. Scott, Cleveland.) I would like to ask what degree of constriction is produced by this band, and secondly, what is the nature of the material encapsulating the kidney.

(Dr. H. L. Jaffe, New York.) I would like to ask Dr. Bell if he constricted one or both veins and whether any changes in the blood followed the use of this method. Did any struggling of the animal have anything to do with the increase in blood pressure? My impression is that in rabbits blood pressure is much lower than those charts show, in the vicinity of 80 or 90.

(Dr. H. Zinsser, Boston.) I was interested in the normal blood pressure of rabbits because there has been a good deal of discussion as to arteriosclerosis present in the ordinary run of laboratory animals. There was a piece of work done not long ago about the changes in the artery due to repeated anaphylactic injury.

(Dr. E. T. Bell, Minneapolis.) Dr. Scott's question: The exact lumen of the vein was not measured but it is reduced to not more than one-sixth of its diameter and we watch it until you can just barely see the blood trickle through. The membrane is an ordinary thin paper condom. Only one vein was occluded. If you occlude both veins you get renal insufficiency and death from uremia.

In support of these views the following observations seem pertinent:

Within five minutes after intratracheal staining the alveoli contain large numbers of phagocytes indicating a readily accessible source of supply. Such a source might conceivably be found in the following locations: (1) cells already in the alveoli; (2) cells in the circulating blood; (3) cells in the permanent structure of the lung. The first source, preëxisting alveolar cells, can be eliminated for two reasons; first, because phagocytes appear too rapidly for cell division to have taken place, and secondly, because the experiments were repeated on guinea pigs, only one day old, whose lungs not being previously irritated by inhaled dust do not contain free phagocytes. The second source, cells in the blood stream, can also be eliminated. The number of available cells remaining in the pulmonary vessels after the circulation has failed is too small to supply all of the free phagocytes which are observed. There remains, then, only some cell in the permanent structure of the lung, as a source of alveolar phagocytes.

The fixed tissue elements which have been suggested as possible origins are alveolar epithelium, capillary endothelium and connective tissue phagocytes or clasmatoocytes. The reactions to neutral red of the first two types in no way resemble those of the alveolar phagocyte. Epithelial cells, studied in thick sections, exhibit only a few fine granules widely scattered throughout the cytoplasm; endothelial cells do not take up the dye at all, unless they are so damaged that the nucleus is stained. Furthermore, there is no evidence of any depletion of these cells after intra-alveolar irritation. Their number remains the same before and after intratracheal injection of neutral red.

The clasmatoocytes of the connective tissue increase in number with the duration of irritation. They are apparently derived from extra vascular monocytes which are in turn produced by stimulation of dormant reticulum cells (Sabin). Clasmatoocytes are found to some extent in the heavy areolar tissues of the trunks, and are very abundant in the alveolar septa. In the latter position they often project into the lumen of the air space between the epithelial cells. They are characterized by great numbers of neutral red granules, varying in size and color, scattered throughout their cytoplasm. After the dye has been injected into the trachea of the recently killed animal, the number of clasmatoocytes in the alveolar septa is notably decreased. This is not so obvious in the living animal where regeneration is constantly taking place. Because of the similarity between the staining of free phagocytes and those in the connective tissues, and because of the depletion of the latter by the intratracheal injection of irritants, we favor this cell as the usual source of alveolar phagocytes.

However, it is not impossible that some of the immature connective tissue monocytes may migrate directly into the air spaces. This form, which is usually smaller than that in the blood, has been found in the alveoli, although it is rare. Its arrangement of neutral red granules in a rosette even more closely resembles that seen in the early free phagocytes than that in the cells of the septa.

Discussion

(Dr. Alfred Plaut, New York.) We have the term desquamation. All the books contain it. One speaks of desquamative pneumonia in tuberculosis and in other infections. I have never been able to find this conception compatible with the normal histology of the human lung. Our alveolar epithelium chiefly consists of extremely thin scales without nucleus and without distinct structure of the protoplasm. Smaller well structured pavement cells with nuclei are in

athletes have no muscular enlargement in spite of exercise. It may depend on family or individual peculiarity. I have often thought of that variety of pathologic condition where the adaptive hypertrophies are common. Sometimes one finds a condition of adaptive hypertrophy without increase in size. I would like to ask Dr. Bell if he thinks that increase in size of the heart would go with that number in order to prove that there was hypertension.

(Dr. Bell.) I cannot answer Dr. Pappenheimer's question as to the relation to senile arteriosclerosis. There are a great many observations to show that sclerosed peripheral arteries have no relation to high blood pressure. It is true that most diagnoses are made on clinical palpation only. We know that senile arteriosclerosis affects chiefly vessels in the extremities, the aorta and sometimes the large vessels of the circle of Willis and the larger branches of the aorta; and as far as we can judge in the cases which we studied there was no greater incidence of high blood pressure than in the normal. With disease of the small arterioles we get high blood pressure whether or not there is also senile arteriosclerosis. I do not have any definite opinion but I think the two conditions are on a different etiologic basis. I do not understand Dr. Marshall's question very accurately.

(Dr. Marshall.) I was referring not to increased heart but to the muscles of athletes. We frequently find athletes in training will show an enlargement of the muscles; after several years of training the visible muscles become markedly larger but the invisible muscles do not particularly tend to become large. This might account for the fourteen cases.

(Dr. Bell, closing.) Those cases are not proved but we must remember the blood pressures were not observed early. We cannot therefore conclude that they are cases of hypertension. There might be some other explanation.

THE ORIGIN OF THE ALVEOLAR PHAGOCYTE STUDIED IN PARAFFIN SECTIONS OF TISSUE STAINED SUPRAVITALLY WITH NEUTRAL RED. Leroy U. Gardner and (by invitation) David T. Smith, Saranac Lake, N. Y.

Abstract. A new method has been devised to preserve supravital staining with neutral red in paraffin sections. Animals are killed by air embolism and the dye is introduced either into the air spaces through the trachea or into the vascular system. This technic has been employed to study the histogenesis of the alveolar phagocyte.

Intravenous staining affects chiefly the cells in the framework of the lung; intratracheally it not only reacts upon these cells, but is sufficiently irritating to provoke a very rapid migration of alveolar phagocytes.

From the character of the cytoplasmic reactions to neutral red it has seemed possible to identify the alveolar phagocyte with the "septal cells" which project above the surface of the epithelium lining the air spaces. These septal cells are in turn classified as a form of clasmatocyte or connective tissue phagocyte.

From the study of this material we have concluded that the septal cells rapidly desquamate because of the presence of the irritating dye in the air spaces. The epithelial cells are not affected but remain in position. Within the alveoli the desquamated septal cells undergo morphologic and physiologic modifications resulting in the formation of typical alveolar phagocytes. New septal cells, replacing those which have migrated from the alveolar walls, are regenerated from connective tissue monocytes which in turn arise from dormant reticulum cells, stimulated by the presence of the irritating dye.

In our case the name adenosis was chosen because it does not prejudice anything and indicates that the condition is not of true neoplastic nature. No overgrowth of muscular tissue was found around the glandular ducts. Probably this disease belongs as a special form into the group of diseases the chief representative of which is the adenomyosis. Our patient was nulliparous.

Discussion

(Dr. A. S. Giordano, South Bend.) The microscopic picture resembled much the endometrial tissue in the so-called adenomyomas. Why could we not explain that on the basis of aberrant endometrium? We find aberrant thyroid in many places and why not aberrant endometrium? Certainly the glandular structure and stroma make it very suggestive of the relationship to endometrium. Going back to the history of this woman, was there curettage? The usual curettage means bleeding. I wonder if this was a type of menstruating endometrium present at that time.

(Dr. Plaut, closing.) I do not consider this tissue identical with endometrium. Endometriosis in the vagina has never been described. The glandular tissue in our case has no relation to endometrium proper. It cannot be a metastatic endometriosis. How can it be explained how it got there? If we should call it ectopic endometriosis we would have to go back to the embryonic state; it could not then be diffuse and restricted to the submucous layer.

the minority. Therefore, it is difficult to trace the large numbers of nucleated cells which are found in the air cells of inflamed lungs to the alveolar epithelium.

(Dr. H. H. Permar, Pittsburgh.) Concerning the question of alveolar epithelium as the source of lung phagocytes, I too would be glad to hear nothing more. Some of us think we have settled that part of the problem, and I wish we might discontinue that part of the discussion. I believe one can see alveolar epithelium in certain pathologic states, tuberculosis, for instance, where there is a definite atelectatic process in the lung. We could discuss the origin of the dust cell all afternoon, but I am not yet willing to give up the endothelial origin. Dr. Gardner's stain does not stain the endothelium. I did not see, in the pictures, the capillaries in the alveolar walls. I should think you would see the nuclei of the cells lining the capillaries there.

(Dr. Gardner.) You can.

(Dr. Permar.) I did not see them in the pictures shown.

(Dr. Gardner, closing.) I might state that in the chronic organizing pneumonia so common in guinea pigs that one occasionally finds areas in which the epithelium has regenerated with the so-called fatty vacuolated type of cell. This type does not take neutral red at all. One can see connective tissue phagocytes lying behind the unstained epithelium.

DIFFUSE ADENOSIS OF VAGINA: A VERY RARE DISEASE. Alfred Plaut, New York City.

Abstract. At the age of 50 years, one year after menopause, a profuse vaginal discharge began. When the patient sought medical help two years later, a tumor-like mass protruded from the vaginal fornix. All the other pelvic organs were normal. Two small pieces were excised, and a diagnosis of adenoma of Bartholin's gland made. The tumor was considered as beginning malignancy. After several weeks the whole vagina together with the uterine cervix was amputated. The entire inside of the vagina was purple-red and showed a number of irregular eroded areas partly with central circular depression. The tissue in the eroded areas appeared granular; the underlying vaginal tissue and the paracolpium were of normal firmness and apparently not invaded by any foreign tissue. The posterior surface of the specimen was smooth.

The microscopic picture is not that of a tumor. Glandular ducts are scattered throughout the whole vagina in the connective tissue between the surface and the muscular coat. The surface epithelium is preserved at a few points only. It is normal squamous epithelium; no connection of it with glandular structures can be seen. The glands show much variation. Often large cavities are seen with smaller ones in the surroundings which obviously are parts of the larger one. Some narrow very long ones are found right under the surface. A low cylindrical epithelial cell prevails. The surrounding connective tissue is infiltrated; this accumulation of cells looks more inflammatory than like cytogenic tissue. Only a slight similarity with endometrial tissue can be noted. The tissue in the fornix is free of glandular structures. There is no connection with cervical glands and no connection with the peritoneal surface epithelium. A derivation from any embryonic remnants is highly improbable in such a diffuse lesion which in a homogenous way involves the whole vagina of an old woman. These glands look as if they had originated at the spot where one sees them now. Two such cases could be found in the literature, one with the diagnosis: diffuse adenoma of vagina, the other one called adenomatosis vaginae.

apex and base systolic and diastolic murmurs were heard. The pulse was of the Corrigan type. The knees, ankles and wrists were somewhat tender and red. Tonsils were enlarged. White blood cells, 7,600, polymorphonuclears 54 per cent. Blood cultures on three occasions remained sterile. The tonsils were removed. He was kept in the hospital for four months.

In October, 1926, he returned complaining of cough and pain in the right chest, chills, fever and headache. His temperature was 104° F. The heart condition had not changed. He was found to have lobar pneumonia, involving the right lower lobe. The consolidation later spread to the right middle and left lower lobes. Blood culture showed no growth of organisms. The white blood cell count of 9,500 with 84 per cent polymorphonuclears on admission increased to 25,000 with 92 per cent polymorphonuclears. During the course of the pneumonia he had pus in his urine. Pneumococcus Type II was recovered from the sputum culture.

About two weeks after entrance an acute infection developed in the left parotid gland which later required incision and drainage. From the gland *staphylococcus aureus* was isolated. Blood cultures after the onset of the acute parotitis remained sterile. The white blood count rose to 30,000 with 90 per cent polymorphonuclears. Following the incision of the gland, the white blood cells quickly dropped to 9,200. The pneumonia, and the parotitis cleared up satisfactorily and when he was discharged from the hospital at the end of two months he showed no signs of cardiac decompensation.

In January, 1927, he came to the emergency ward complaining of pain in both flanks. No cause for this pain could be discovered. At this time he also had acute pharyngitis.

Three weeks later he again returned because of a "pounding" pain in the chest and abdomen, unrelated to respiration. He again had acute pharyngitis. He was admitted to the hospital. The heart condition had not changed. A single petechia was discovered in the conjunctiva of the right eye. The fingers were not clubbed. The lower border of the liver was 3 cm. below the costal margin. The spleen could not be palpated. The white blood cells numbered 9,100 with 65 per cent polymorphonuclears.

The pain became localized to the precordial region; a pericardial friction rub became evident ten days after he entered the hospital, and at about the same time he had an attack of polyarthritis. The white blood count rose to 28,000 with 86 per cent polymorphonuclears. His temperature frequently reached 103° F.

Blood cultures were made on three occasions. Two of the cultures were incubated for four weeks, the other for six weeks. No growth occurred in any of them. He died thirteen days after entrance in the hospital.

HEART

Gross Examination: The heart is greatly enlarged, weighing with a portion of the pericardial sac, 740 gm. The parietal pericardium over several areas is bound by dense adhesions to the heart. Elsewhere the pericardial surfaces are covered with a thick, shaggy, fibrinous exudate. All chambers are enlarged. The right and left ventricles are hypertrophied. Along the line of closure of the septal

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STUDIES IN THE PATHOLOGY OF RHEUMATIC FEVER*

TWO CASES PRESENTING UNUSUAL CARDIOVASCULAR LESIONS

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The more closely one studies material from cases of rheumatic disease the more does it become apparent that the lesions of the vascular system are more diverse and more widely distributed than is generally supposed. The cases here reported illustrate this point and add several new observations to previous descriptions of the pathology of rheumatism. These new features of interest are, in brief, an aortitis with peculiar characteristics not heretofore described and a specific type of panarteritis affecting the larger arteries (coronaries, renal, superior mesenteric and coeliac axis). The absence of complicating lesions and infections, the youth of the patients and the typical clinical courses add significance to the findings.

CASE I

F. L. (History 62760, Necropsy 9867), white male, age 15 years.

Family History. Irrelevant.

Past History. Negative.

Present Illness: Onset in 1922 with an attack of sore throat, followed after a week by swollen, painful, tender joints of lower extremities and hands. During the next two years he had eight to ten similar attacks of polyarthritis, usually preceded by sore throat. In 1923 he began to have dyspnea, orthopnea and palpitation.

He was first admitted to the Presbyterian Hospital in April, 1925, complaining of swollen, red and painful joints, shortness of breath and fever which had continued for one month. At this time his temperature was 103.6° F; he was somewhat cyanotic and definitely orthopneic. His heart was greatly enlarged, the left border reaching to the axilla. The heart rate was rapid. At the

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philes and polymorphonuclear neutrophiles, often localized to certain areas in the greatly thickened endocardium (Fig. 5).

On the surface, the single row of flattened cells has been supplanted in many places by a peculiar new tissue, which merits more detailed description, since the same sort of tissue is found lining many of the peripheral vessels. The cytologic composition of this tissue is difficult to analyze. It consists of spindle-shaped cells directed for the most part perpendicularly to the surface. Their nuclei are pycnotic, elongated, often somewhat angular, or drawn out into strands of odd shape. The outlines of individual cells are indefinite. The groundwork is formed by an indistinct fibrillar material, and by the basophilic cytoplasm of the large cells. In the interstices are leucocytes, both polymorphonuclear and occasionally eosinophiles. Many of the nuclei are too distorted to be readily identified (Fig. 6).

The relation to the elastic fibers is somewhat variable. The fibers may be found on either side of this new tissue, and fine fibrillae, often cut in cross-section, run between the individual cells (Fig. 7).

The tissue does not always lie upon the surface, but may be covered by a stratum of fibro-elastic tissue running parallel to the surface.

Very interesting lesions are present also in the endocardium of the left ventricle. In places these have the usual structure and arrangement of Aschoff nodules. Thus Fig. 8 shows a rosette of Aschoff cells disposed about a mass of necrotic collagen. But a more diffuse reaction is found over large areas of endocardium (Figs. 9 and 10). Dense collections of nuclei belonging to polymorphonuclears, lymphoid cells, large mononuclears with vesicular nuclei (endotheliocytes?) and other nuclei which cannot be identified because of their distorted shape and pycnotic staining, occupy the loose fibro-elastic tissue of the endocardium and project irregularly upon the surface. There is no thrombus or vegetation superimposed; the overlying endothelium is intact in some places, but in others it is lost. The cells taking part in this reaction are identical with those in the auricular endocardium, although their arrangement is slightly different. These infiltrations were the cause of the yellow flecks seen in the ventricular endocardium.

and infundibular leaflets of the tricuspid valve are small areas of roughening. The pulmonic valves are normal. In the left auricle, the endocardium above the posterior leaflet of the mitral valve presents ridges and furrows; these run roughly perpendicular to the valve ring and extend also to the orifice of the auricular appendage. The area so involved is 8×5 cm. Both leaflets of the mitral valve are thickened and are fused together so that the opening measures only 5.2 cm. On the posterior leaflet along the margin in two places are several small yellowish grey, tough vegetations. The chordae tendineae are markedly thickened. The endocardium of the left ventricle has numerous yellow flecks in it. Near the apex of the posterior papillary muscle is an endothelial pocket pointing upward toward the auricle. Below the anterior and left posterior aortic leaflets the endocardium is thrown up into folds, one of which forms a small pocket, directed toward the apex. The aortic leaflets are thickened. The free border of the right posterior leaflet of the valve is rolled toward the sinus side. There is some fusion between the anterior and right posterior leaflets. Small yellowish grey vegetations are present on the free margin of the right posterior leaflet. The myocardial fibers are coarse. There is no apparent scarring.

Microscopic Examination: The histologic structure of the vegetations on the mitral and aortic valves is that characteristic of rheumatic verrucae — hyaline, eosin-staining masses, into the base of which large fibroblast-like cells are growing (Fig. 1). There is no polymorphonuclear reaction, and no bacteria are found. The valve is greatly thickened, vascularized, and contains occasional mononuclears and eosinophiles and a few imperfectly formed Aschoff bodies surrounding areas of fragmentation and swelling of the collagen (Fig. 2).

The myocardial lesions are intense and characteristic. Throughout all the blocks examined, one sees typical lesions in the vicinity of small branches of the coronary arteries (Fig. 3). Since these present all the well-known features of Aschoff lesions, they need not be described in detail. The fibrinous pericarditis is shown in Fig. 4.

The auricular lesions conform in their histologic details to the descriptions given by MacCallum¹ and by VonGlahn² in previous papers. They may be summarized as consisting of band-like necroses of collagen about which are palisades of basophilic-staining cells, collections of distorted and fragmented nuclei, with eosino-

ally two large vesicular nuclei containing a central dense clump of chromatin (Figs. 13 and 14). In their arrangement and staining reaction, these cells are identical with those so characteristically present in the auricle. Where these cells are more sparsely arranged, they assume very irregular shapes. The nucleus is often lobate or bent upon itself, and long plasmatic processes can be traced from the cell body into the crevices of the fibrillar matrix.

The medial lesions in the areas below the intimal plaques are diffuse, but definite. They are best brought out in sections prepared by the Weigert-Van Gieson methods. Perhaps the most striking change is complete loss of muscle cells over large areas, so that only the collagen and elastic fibers persist. This change is foreshadowed in the hematoxylin-eosin preparations in which the muscle nuclei in the corresponding areas have largely disappeared. The elastic fibers are thinner, less wavy and less well stained than in the better preserved regions of the media. In places there is actual rupture or fragmentation.

Toward the outer third of the media are encountered perivascular lesions which are like those described in previous papers. They are unusually numerous and many of the cells found in the vicinity of the nutrient vessels have as definitely the character of "Aschoff cells" as in any case which we have studied (Fig. 11).

Blocks taken through the large plaques above the aortic leaflets bring out certain additional features. The intimal plaque itself is composed of a rather loose tissue made up of irregular cells tending to a vertical orientation and separated by an indefinite fibrillar stroma. At the margin of the plaque, the endothelium, which in the normal portion of the aortic wall consists of a flattened layer of single cells, becomes dissociated; the individual cells assume a polyhedral form and appear to migrate into the subjacent stroma. Occasionally clefts containing red blood cells are bordered by such polyhedral cells (Figs. 15 and 16). In certain sections, the clefts appear to communicate with the lumen of the vessel.

This peculiar tissue resembles in no wise the tissue which composes the ordinary atheromatous plaque. Sections stained with Scharlach R show only occasional cells containing finely divided fat, which with the polarizing microscope is not anisotropic. There are no free lipoid masses, no large foamy fat-containing cells and no cholesterol crystals. One interesting field is illustrated in Fig. 17.

AORTA

In previous papers dealing with rheumatic aortitis, Klotz³ and the writers⁴ described lesions which followed the distribution of the vasa vasorum into the outer portion of the media. The present case shows abundant lesions of this sort (Fig. 11). But there is, in addition, a pathologic process easily differentiated from the common form of atherosclerosis, which affects predominantly the *intima* and the *subjacent portions of the media*. The rheumatic origin of these lesions will become clear from the analogies to the changes in the auricular endocardium.

Gross Examination: The aorta has the appearance illustrated in Fig. 12. In each of the sinuses of Valsalva are small ridges or plaques in the intima. These are pale brownish in color, glistening and translucent. Above the junction of the anterior and left posterior leaflets of the aortic valve is a larger similar plaque which reaches to the orifice of the left coronary artery. Above the anterior and right posterior aortic leaflets, and separated from them by apparently normal aorta, is a brownish, translucent intimal plaque having a delicately ridged surface as though small areas had become confluent. In nearby portions of the intima are several small, rounded, or oval areas of similar appearance.

Near the margin of the large plaque are a number of narrow atheromatous streaks; these are opaque, orange-yellow and dull.

The pale brown elevations are very similar in appearance to the lesion of acute rheumatic auricular endocarditis, and are in sharp contrast with the dull opaque orange-yellow atheromatous lesion, and the grey glistening wrinkled plaque of syphilitic aortitis.

Because of the restrictions imposed upon the necropsy, the remainder of the thoracic aorta was not removed. In the abdominal portion are a few characteristic atheromatous streaks. Near the bifurcation the wall of the aorta seems distinctly thickened as compared with the vessel wall in the region of the diaphragm.

Microscopic Examination: The microscopic changes affect all the elements of the intima, and in many places the subjacent media.

A block was taken through a translucent plaque shown at the upper cut edge of the ascending aorta. Here are found bands of non-nucleated fibrillar material in the intima, bordered by rows of deeply staining cells with basophilic cytoplasm and one or occasion-

mass of infiltrating cells, which have replaced the muscular elements and pushed up the intact overlying endothelium into the lumen. The appearance of this lesion is shown in Figs. 24 and 25.

COELIAC AXIS AND SUPERIOR MESENTERIC ARTERIES

It was discovered in the fixed specimens of duodenum and pancreas that both these arterial trunks were very greatly thickened. The lumen was much reduced in diameter and in the case of the superior mesenteric artery, it appears in the form of a tortuous cleft because of the presence of new tissue in the intima (Fig. 26). The walls are creamy white and very greatly thickened in comparison with those of a normal vessel cut in the same situation in another individual of about the same age.

Microscopically, all the coats are affected by an inflammatory and productive reaction of great intensity but presenting certain features which correlate the lesions with those found in other portions of the cardiovascular system.

The intima is much thickened by the new formation of a loose fibrocellular tissue. The overlying endothelium is preserved. In places the superficial areas are quite cellular and the arrangement and morphology of the cells resemble that in the new tissue covering the aortic plaques. In other areas the cells are few and far apart, being separated by a pink, more or less hyalinized ground substance.

The internal elastic lamella is highly irregular, being split into numerous laminae, some of which lie in the superficial portion of the plaque, just over the new tissue above described; other laminae lie between the intimal plaque and the media. The area in which the cells are sparse and the ground substance more or less hyalinized contains virtually no elastic fibers. Elsewhere in addition to the circumferentially disposed lamellae, there are numerous delicate fibrils running in all directions. In several areas just internal to the innermost layer of the internal elastic lamella, these cellular foci are composed largely of distorted polymorphonuclear cells (Fig. 27).

The chief lesion in the media consists in the presence of polymorphonuclears, scattered individually between the muscle fibers, but occasionally forming more compact aggregates (Fig. 28). In some areas, there is a local loss of muscle nuclei, their place being taken by a fine fibrillar ground substance. The muscle nuclei, especially

Here the internal elastic lamella is split into two distinct layers; the upper one is intact, lying immediately beneath the stratum of intimal tissue described. The lower lamina in places is interrupted by patches of scar tissue, about which are grouped irregular vertically disposed cells. This gives indisputable proof of the cicatricial character of these intimal lesions.

The adventitia is very greatly thickened in all blocks examined, and particularly in those taken from the ascending arch in the region of the plaques, and from the lower abdominal aorta. The nutrient arteries have thick walls; the different coats are not easily distinguished. The lumen is often narrowed (Fig. 18) and in some of the large vessels lined with a layer of new tissue closely resembling that in the intima of the aorta. The elastic fibers are frayed and reduplicated and the muscle fibers are separated by connective tissue and young fibroblasts.

In one nutrient vessel in the abdominal portion, there is a focal collection of large cells resembling an Aschoff nodule (Fig. 19).

CORONARY VESSELS

1. A block was taken through the beginning of the left coronary artery close to its orifice. The changes are similar to those seen in the aortic plaque; the intima is represented by a loose tissue which appears to be derived from the endothelium itself, together with a few wandering cells, including a fair number of polymorphonuclears (Fig. 20).

2. A large branch of a coronary artery shows an elevated parietal thrombus projecting into the lumen. This is composed of a mass of fibrin, the filaments of which run vertically. Entangled in the fibrinous net are numerous deeply stained nuclei, many of which appear to belong to polymorphonuclears. A few leucocytes are seen between the muscle fibers beneath the thrombus (Figs. 21 and 22).

3. A lesion is present about a small capillary which resembles that described and pictured in a previous paper.⁵ The vessel over a small area is surrounded by a mass of fibrin which infiltrates the surrounding tissue. At the margin of the fibrin there are many Aschoff cells and a few polymorphonuclear leucocytes. The lumen is not occluded by thrombus and the endothelium is intact (Fig. 23).

4. A vein is cut longitudinally. One side of the wall shows a normal structure; the other is lifted up into irregular prominences by a

32. Beneath a small atheromatous plaque composed of large lipid-containing cells, with a few smaller lymphoid elements, are seen rows of large polyhedral basophilic cells, vertically directed about bands of non-nucleated fibrillar material. The other lesion does not lie beneath an atheromatous plaque. The deeper portion of the intima and the subjacent media are occupied by dense accumulations of cells of two types: (1) large polyhedral elements with vesicular nuclei, like those in the other lesion and (2) deeply staining nuclei, some of which obviously belong to polymorphonuclears; others are distorted beyond recognition, the chromatin being drawn out into threads with bulbous ends (Figs. 33 and 34). This combination is exactly like that so characteristically found in the auricular lesions, as may be seen in Fig. 5.

In the superior mesenteric artery of this case, studied in the fixed museum specimen, there are seen with the stereoscopic microscope gelatinous reddish elevations projecting into the lumen. Sections from these areas show lesions of the intima like those noted in the previous case. The most striking feature is the presence of the distorted, deeply staining nuclei in a zone which includes the intima and the media immediately outside of the internal elastic lamella. Some of these nuclei are derived from migrating polymorphonuclear leucocytes since similar cells in the auricular lesion have been found to give the oxidase reaction; others, however (those which are more elongate), appear to belong to large mononuclear wandering cells as they show no oxidase granules. The lesions are not present throughout the circumference; in some fields, the intimal tissue shows a normal structure. The internal elastic lamella is split into two or several layers and is swollen. A few leucocytes with distorted nuclei may be found in all portions of the media. The adventitia is free from striking changes. A few loose perivascular lymphoid accumulations are present.

CONCLUSIONS

It has not been thought necessary to review again the literature dealing with rheumatic vascular disease, since this has been adequately covered in previous papers and in the recent admirable summary by Sacks.⁶

We have found a few references to the occurrence of verrucous lesions on the intima of the aorta and, in one instance (Heydloff⁷),

those in the vicinity of these rarefied areas, are distorted and vesicular rather than rod-shaped.

The adventitia is represented by dense collagenous connective tissue radiating into the surrounding fat. Between the bundles of fibers are numerous capillaries characterized by very swollen endothelium, almost occluding the lumen. In addition there are many small lymphoid cells, polymorphonuclears and large mononuclears with basophilic cytoplasm and processes extending into the crevices of the tissue (Fig. 29).

Frozen sections stained with Scharlach R show only a few cells in the intima containing finely divided fat. As in the other lesions, this is not anisotropic.

RENAL ARTERY

In one of the branches an early intimal lesion of similar nature is encountered (Fig. 30).

CASE II

We have found in our material another clearcut example of this type of aortic involvement.

The case was that of a negro boy, age 14 (History 60354, Necropsy 9529) who had had frequent attacks of sore throat until his tonsils were removed, but never polyarthritis. A year before entering the hospital he had an attack of precordial pain, dyspnea and fever, following which he was short of breath on exertion. Three weeks before admission he began to have substernal pain which spread to the precordium. Later he had fever and sweats, and became very dyspneic and orthopneic. For four days his feet had been swollen. At the apex of the heart a presystolic and a systolic murmur were heard; at the base a diastolic murmur was present. His pulse was collapsing. He died four days after entering the hospital.

The pericardial sac is everywhere bound to the heart by fibrous adhesions. Typical verrucae are present on the tricuspid, mitral and aortic valves. The left auricle presents the characteristic picture of rheumatic involvement of the endocardium, and the case has been reported in that connection (Case VI).² In the myocardium are numerous Aschoff bodies.

The aorta grossly shows only what appears to be early atheroma in the form of narrow orange colored streaks along the posterior wall of the descending thoracic and abdominal portions.

A section taken from the midthoracic portion shows two lesions, differing somewhat from one another but preserving the now familiar features as seen in the auricle. One of these is shown in Figs. 31 and

It is not maintained that this difference in type of reaction is of fundamental importance; it may be in part dependent upon the peculiarities of the structural arrangement of the tissue in which the reaction is occurring.

It seems to us, however, of importance to recognize the fact that the pathology of rheumatic infection does not begin and end with the Aschoff nodule. Experience has taught us to recognize as equally distinctive the more diffuse reaction whether it occur in the valves, endocardium, aorta or the smaller visceral vessels.

We are indebted to Dr. Walter W. Palmer for permission to transcribe the clinical records, and to Mr. Alfred Feinberg for the drawing.

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DESCRIPTION OF PLATES

PLATE 146

- FIG. 1. Necropsy 9867. Rheumatic endocarditis of aortic valve. (H. and E. stain.)
- FIG. 2. Necropsy 9867. Aortic valve: Necrosis of collagen with beginning accumulation of Aschoff cells. (H. and E. stain.)
- FIG. 3. Necropsy 9867. Aschoff body in myocardium. (H. and E. stain.)
- FIG. 4. Necropsy 9867. Rheumatic pericarditis. Exudate of fibrin with hemorrhage and early organization.

in the innominate and carotid arteries. In one of the best described cases, that of Barbacci,⁸ these lesions began 1 cm. above the sinus of Valsalva and histologically were not unlike the organizing verrucae of rheumatic endocarditis. Bacteria could not be demonstrated in them. The heart was normal.

It is quite possible that some of the cases were rheumatic in origin though no reference is made to this possibility by the authors. Neither are the histologic descriptions sufficiently detailed to justify any definite conclusions. It must be remembered, however, that these papers antedated the recognition of the specificity of the lesions in rheumatic cardiac disease.

While many of the older writers, and particularly French authors, have ascribed to the "rheumatic virus" an important place in the causation of arterial disease, the connection was based upon clinical inference rather than histologic specificity. The recent studies upon the auricular lesions have made it apparent that the rheumatic infection may be evidenced by a diffuse response as distinctive in its cellular composition and arrangement as is the Aschoff nodule or the endocardial verruca.

It is therefore not surprising that one may find this specific type of reaction not confined to the heart, but taking place also in the lining of the aorta and the larger arteries. The detailed study of these cases brings evidence of this fact. Such evidence is of necessity indirect and based chiefly upon the analogy of the lesions in the blood vessels with those admittedly of rheumatic origin occurring in the auricular endocardium. Until the specific agent shall have been finally identified and similar lesions experimentally produced, this is as far as one can go.

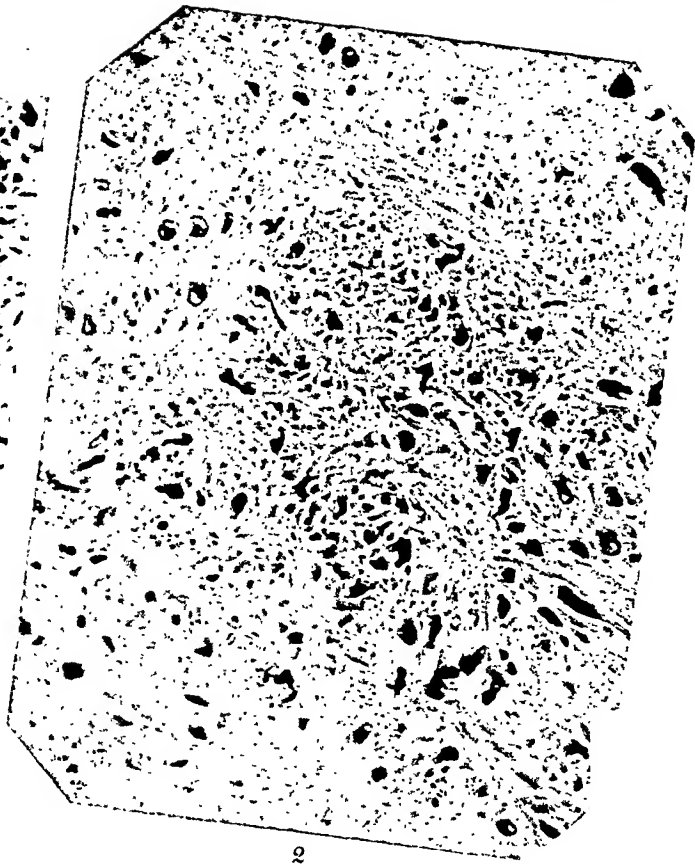
The lesions which have been depicted in the aorta are, in these cases, not mere extensions from the aortic valves, since they occurred at a distance, indeed in the second case, in the midthoracic portion. The aortitis is of two types, which may be associated or occur discretely, and which probably corresponds to the route of infection. When this takes place from the lumen, there is produced a diffuse reaction in the intima and subjacent media, comparable to that which so frequently affects the auricular endocardium. On the other hand, infection reaching the aortic wall via the vasa vasorum produces a focal perivascular reaction, more closely analogous to the Aschoff nodules about the small branches of the coronary vessels.

PLATE 147

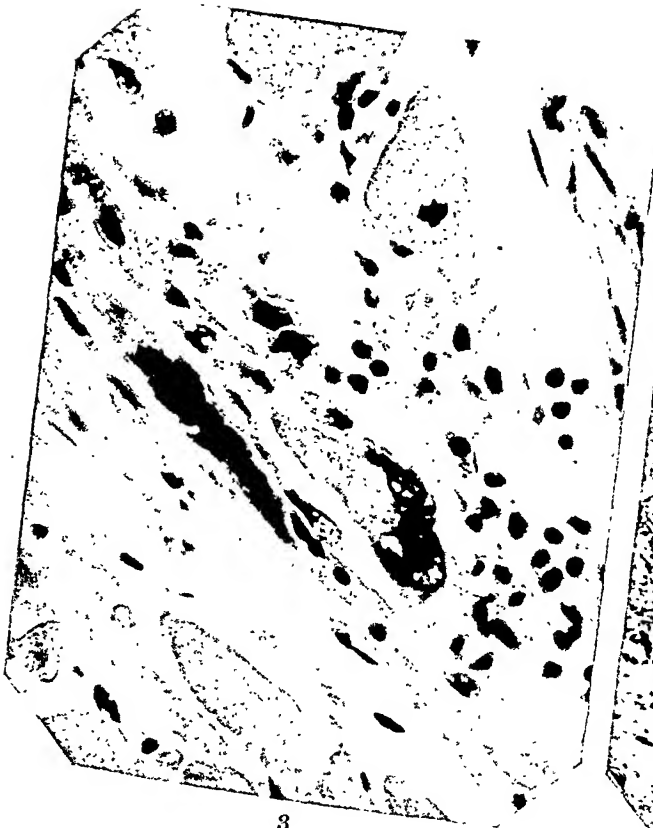
- FIG. 5. Necropsy 9867. Acute rheumatic endocarditis of left auricle. Band of necrotic collagen with palisade of basophilic cells on one side; on the other side, a collection of cells, consisting of polymorphonuclear neutrophiles, eosinophiles, and cells with distorted nuclei. (H. and E. stain.)
- FIG. 6. Necropsy 9867. Rheumatic endocarditis of left auricle. New tissue composed of cells directed perpendicularly to surface, polymorphonuclear leucocytes and spindle cells. Nuclei of cells distorted and pycnotic. The new tissue penetrates through gaps in the elastic fibers. (H. and E. stain.)
- FIG. 7. Necropsy 9867. Rheumatic endocarditis of left auricle. Consecutive sections. Photographs of corresponding fields. (A) Layer of new tissue composed of cells as described in Fig. 6. (H. and E. stain.) (B) The new tissue lies internal to the elastic fibers. (Elastic tissue and Van Gieson stain.)



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Pappenheimer and VonGlahn

Pathology of Rheumatic Fever

PLATE 148

FIG. 8. Necropsy 9867. Aschoff body in endocardium of left ventricle. (H. and E. stain.)

FIG. 9. Necropsy 9867. Rheumatic endocarditis of left ventricle. Acute diffuse reaction. Many polymorphonuclears, lymphoid cells, and large mononuclear cells. (H. and E. stain.)

FIG. 10. Same as Fig. 9. High power.

FIG. 11. Necropsy 9867. Rheumatic aortitis. Collection of cells about penetrating vessel in media. Some of the cells are small mononuclears, others are typical Aschoff cells. (H. and E. stain.)

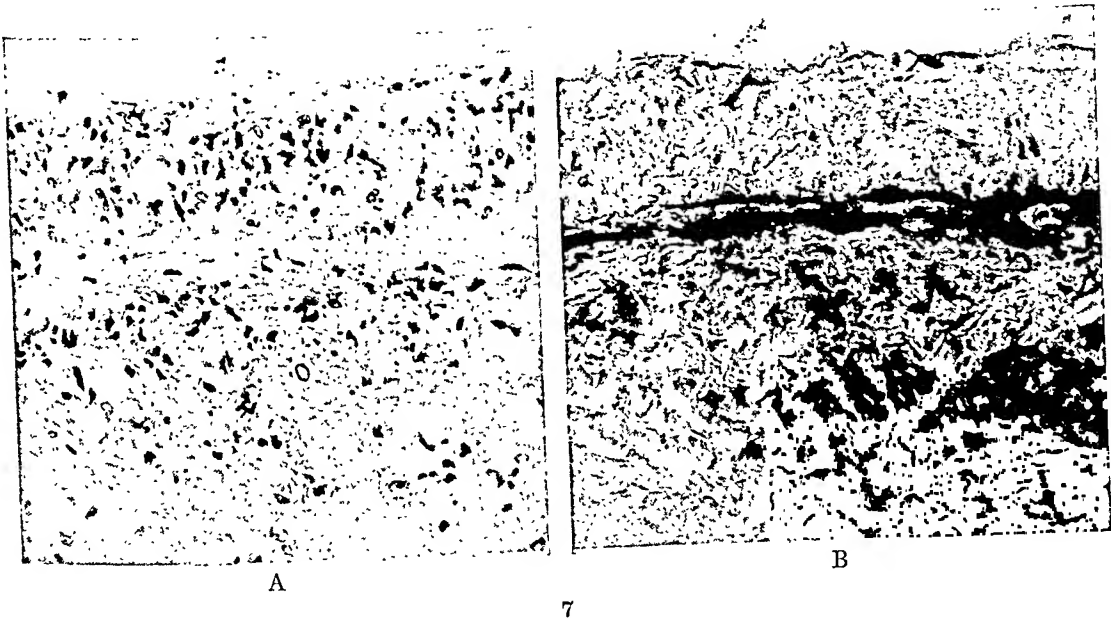
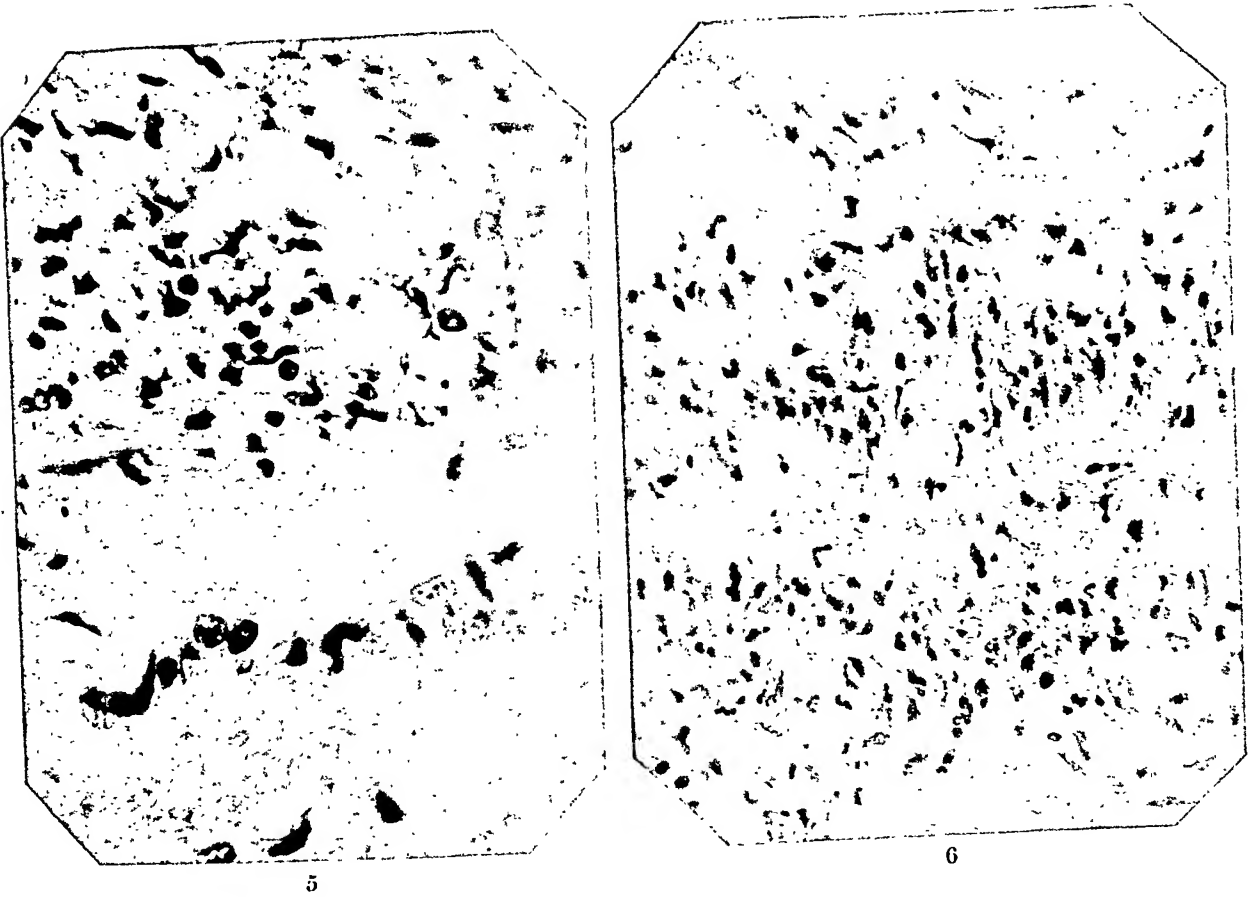
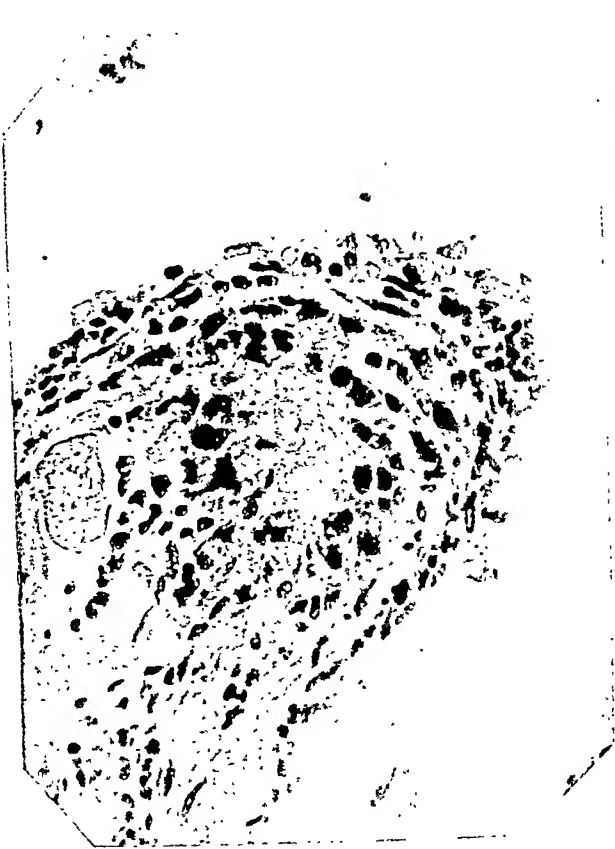


PLATE 149

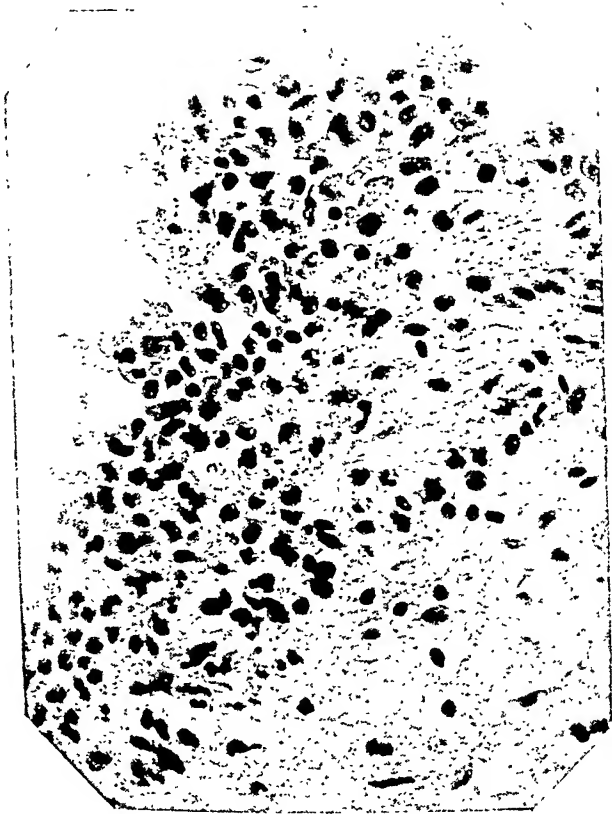
FIG. 12. Necropsy 9867. Rheumatic aortitis. Translucent pale brown plaques in intima in sharp contrast to atheromatous streaks nearby. Rheumatic endocarditis of aortic valve. Thickening of ventricular endocardium beneath aortic valve. Anterior leaflet of mitral valve and chordae tendineae thickened. Rheumatic pericarditis.



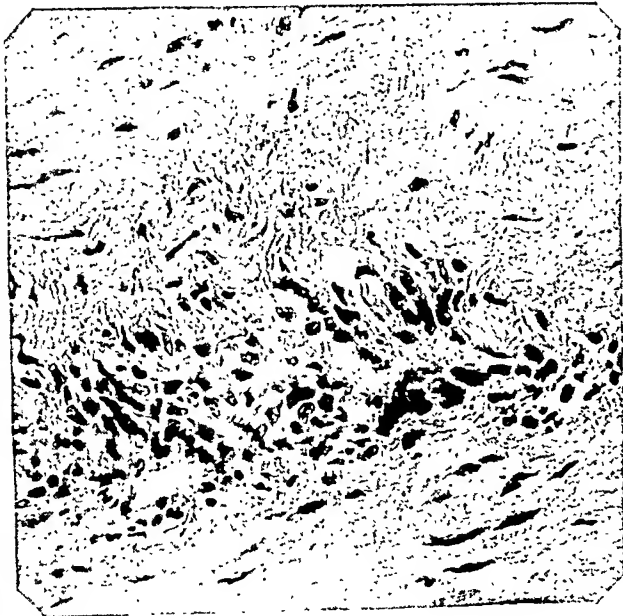
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PLATE 150

FIG. 13. Necropsy 9867. Rheumatic aortitis. Bands of non-nucleated fibrillar material in the intima, bordered by rows of cells with basophilic cytoplasm. (H. and E. stain.)

FIG. 14. Same as Fig. 13, high power.

FIG. 15. Necropsy 9867. Rheumatic aortitis. Consecutive sections. Photographs of corresponding fields. (A) Intimal plaque composed of loose tissue made up of irregular cells separated by fibrillar stroma. Small clefts in the new tissue. In the adjacent media, most of the nuclei have disappeared. (H. and E. stain.) (B) The intimal plaque is internal to the elastica interna. Rupture and fragmentation of the elastic fibers in adjacent part of media. (Elastic tissue and Van Gieson stain.)

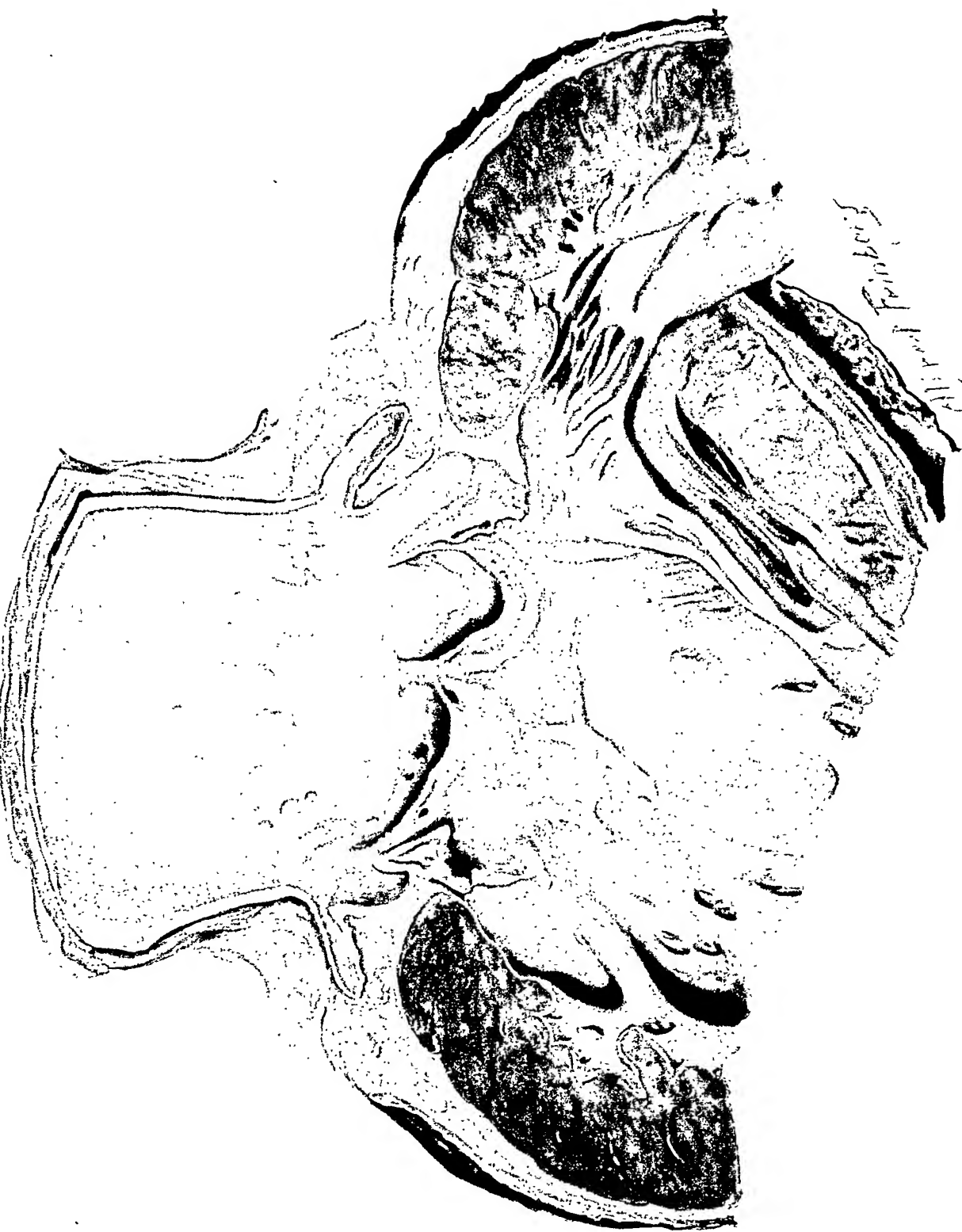
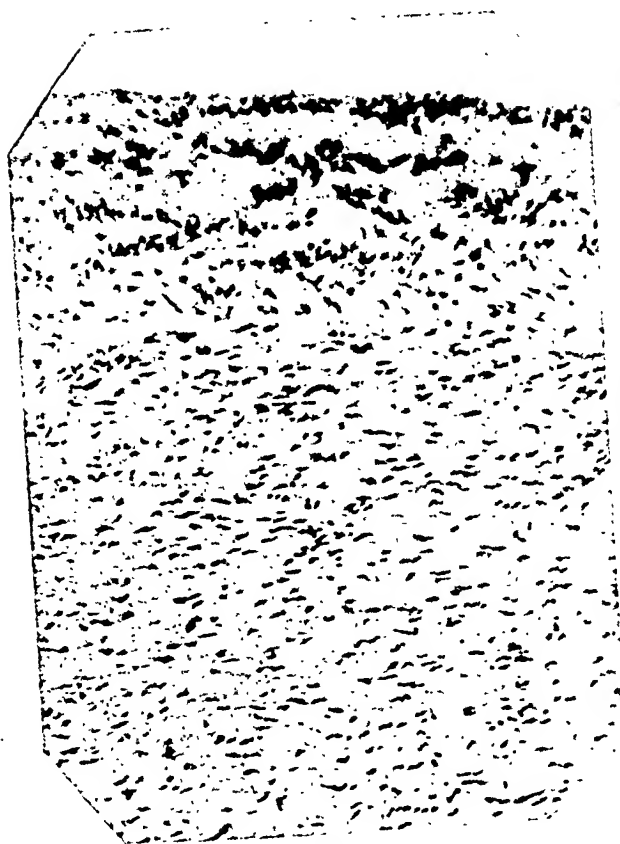


PLATE 151

FIG. 16. Rheumatic aortitis. High power of a field of Fig. 15, A.

FIG. 17. Necropsy 9867. Rheumatic aortitis. Layer of new tissue as described in Fig. 15; internal elastic lamella split into two layers. The inner layer is intact and lies immediately beneath the new tissue; the outer layer is interrupted by patches of scar tissue. (Elastic tissue and Van Gieson stain.)

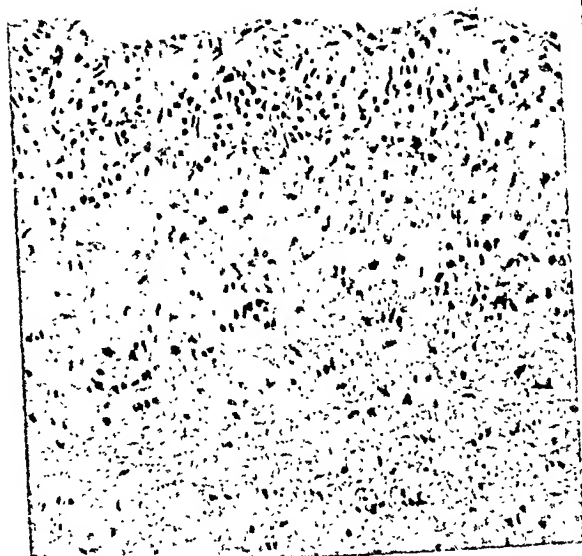
FIG. 18. Necropsy 9867. Rheumatic aortitis. Great thickening of adventitia. Nutrient artery thick-walled and lumen narrowed. Thoracic aorta. (H. and E. stain.)



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A



B

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PLATE 152

FIG. 19. Necropsy 9867. Section from abdominal aorta. Nutrient vessel with focal collection of large cells in its wall. One of the cells contains two nuclei. (H. and E. stain.)

FIG. 20. Necropsy 9867. Left coronary artery. New tissue in intima, apparently derived from the endothelium together with few wandering cells and polymorphonuclears. (H. and E. stain.)

FIG. 21. Necropsy 9867. Coronary artery. Parietal thrombus. (H. and E. stain.)

FIG. 22. Same as Fig. 21, high power.



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PLATE 153

- FIG. 23. Necropsy 9867. Capillary in myocardium. Fibrin on either side of vessel, with Aschoff cells and polymorphonuclears. Lumen of capillary not occluded by a thrombus. (H. and E. stain.)
- FIG. 24. Necropsy 9867. Vein in myocardium. Vessel wall infiltrated with polymorphonuclears and large basophilic mononuclear cells; a few cells have distorted nuclei. Endothelium swollen but intact. (H. and E. stain.)
- FIG. 25. Same as Fig. 24, high power.
- Fig. 26. Necropsy 9867. Superior mesenteric artery. Thickening of adventitia and intima. Lumen narrowed. (Elastic tissue and Van Gieson stain.)



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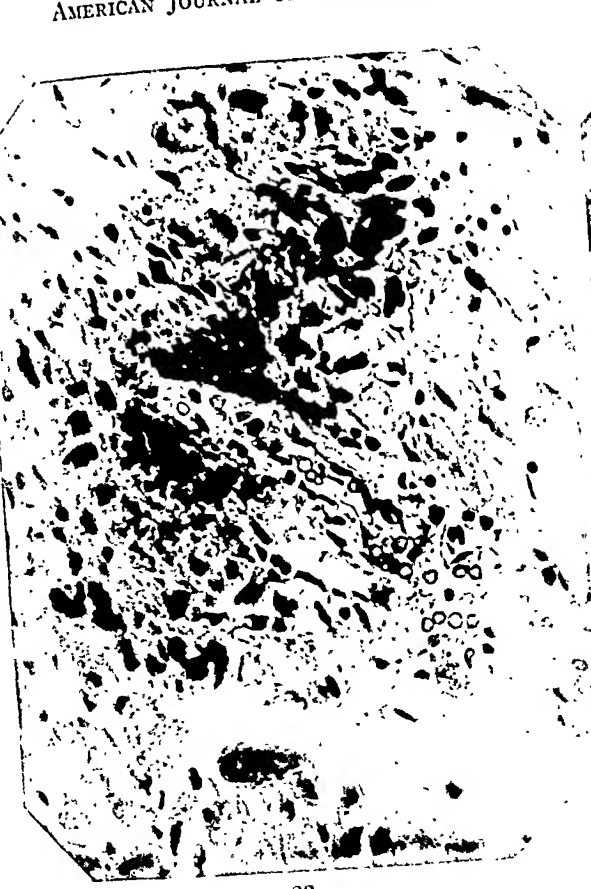
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PLATE 154

- FIG. 27. Necropsy 9867. Coeliac axis. Intima thickened by a loose fibro-cellular tissue. Accumulation of polymorphonuclear leucocytes internal to the elastic interna. Many of the leucocytes have distorted nuclei. (H. and E. stain.)
- FIG. 28. Necropsy 9867. Coeliac axis. Infiltration of media with polymorphonuclears. (H. and E. stain.)
- FIG. 29. Necropsy 9867. Coeliac axis. Polymorphonuclear infiltration of media. Adventitia thickened by dense collagenous connective tissue, in which are capillaries, small lymphoid cells, polymorphonuclears, and large mononuclear cells. (H. and E. stain.)
- FIG. 30. Necropsy 9867. Renal artery. Few polymorphonuclears in intima. (H. and E. stain.)



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PLATE 155

FIG. 31. Necropsy 9529. Rheumatic aortitis and early atheroma. Beneath the atheromatous area are bands of non-nucleated fibrillar material surrounded by large basophilic cells. (H. and E. stain.)

FIG. 32. High power of a portion of field shown in Fig. 31. Large basophilic cells about bands of non-nucleated fibrillar material.

FIG. 33. Necropsy 9529. Rheumatic aortitis. Infiltration of intima and adjacent portion of media with large mononuclear cells and polymorphonuclears. The nuclei of many of the cells are greatly distorted. (H. and E. stain.)

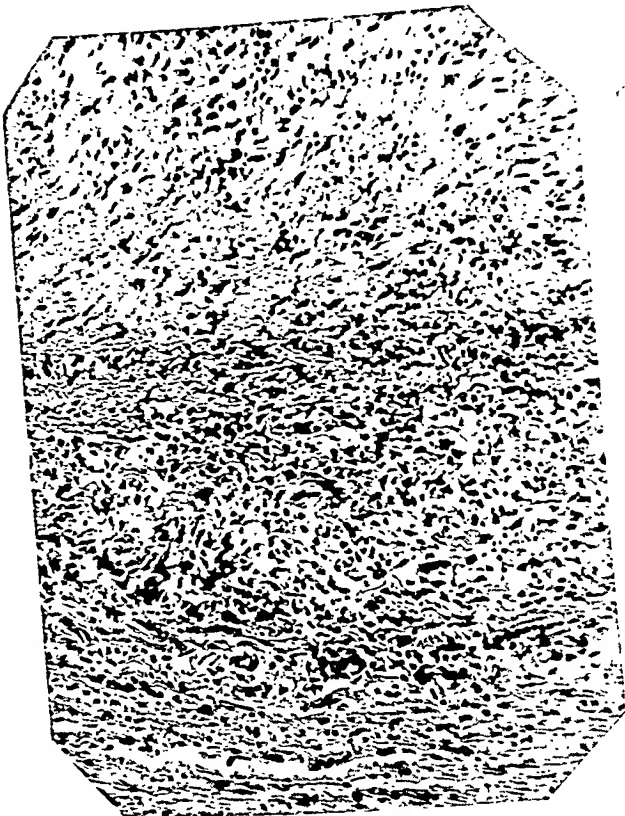
FIG. 34. Same as Fig. 33, high power.



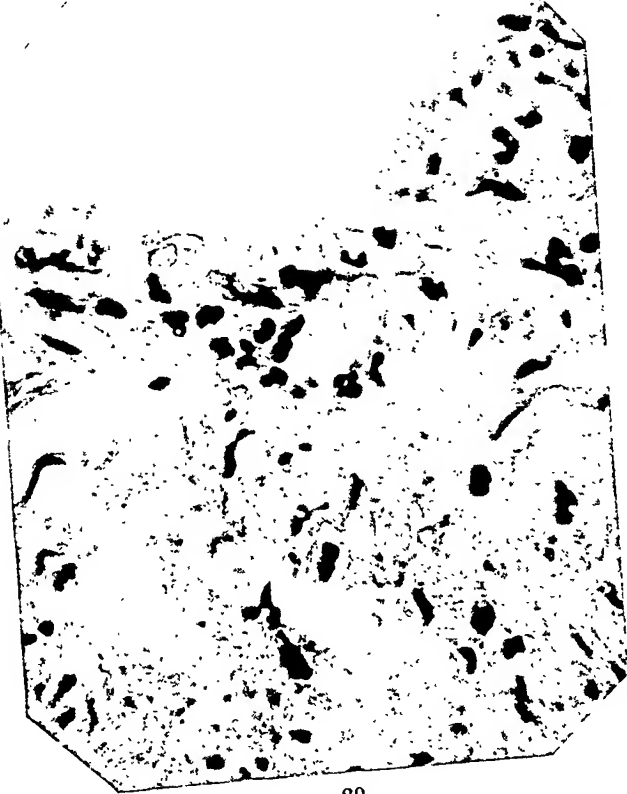
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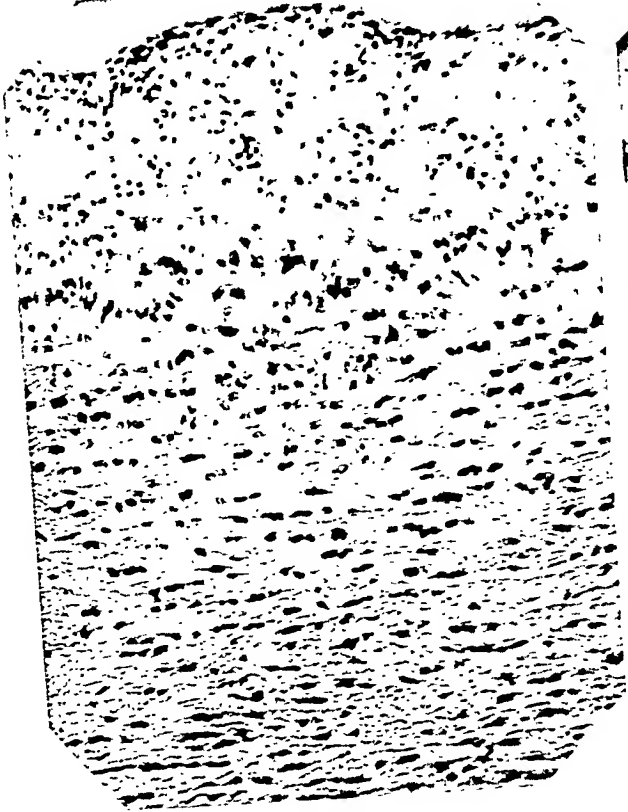
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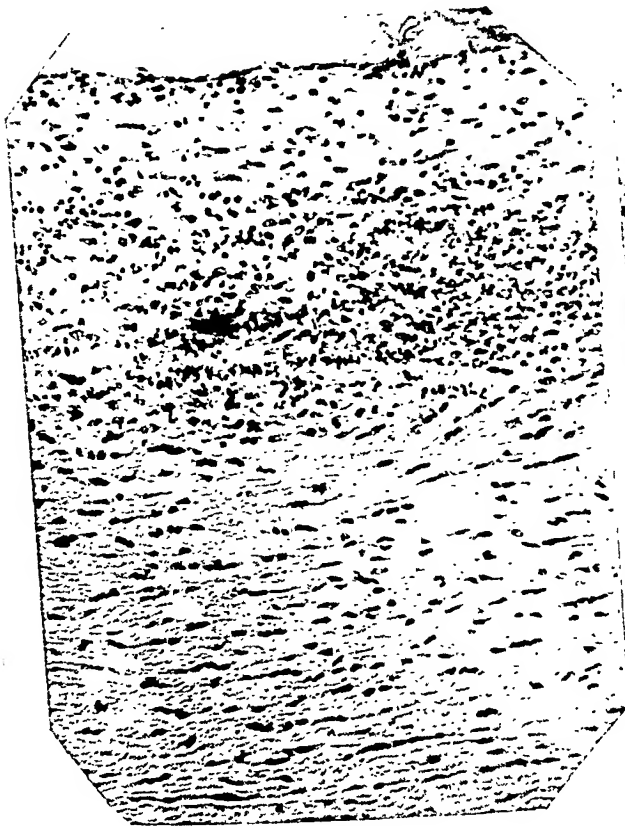
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- (2) Extensive hypertrophy and marked dilatation of the heart, especially of the right side, with fatty degeneration of the myocardium. (Weight 50 gm. in fixed condition; circumference at base, 14 cm.; transverse diameter, 4.6 cm.; base to apex, 6.5 cm.)
- (3) Chronic passive congestion of the lungs, liver, and spleen.
- (4) Partial fetal atelectasis of both lungs.
- (5) Mongolian facies.
- (6) Aplasia of the third phalanx of the left fifth finger.

DESCRIPTION OF THE HEART

The heart is large and generally dilated; its apex is rounded. The dilatation is more marked on the right side, especially in the atrium and auricular appendix. The left auricular appendix is unusually small and appears rudimentary.

When the heart is opened there appears a gross defect of the upper part of the interventricular septum and of the lower part of the interatrial septum, as well as an undivided atrioventricular canal, all four chambers thus communicating freely. The interventricular septum is totally defective in its membranous portion, presenting instead, in this region, an aperture of communication between the ventricles. The muscular septum, the upper margin of which is bow-shaped with its convexity downward, forms the lower boundary of the interventricular aperture. The anterior margin of the aperture curves sharply upward to the right semilunar aortic cusp. Anterior to the aperture the ventricular septum is normal and forms the posterior wall of the conus. The inferior margin of the aperture slopes in a gentle curve upward and backward to the posterior wall of the left atrium. From this region there extends into the aperture a finger-like process which will be described later. The extent of the aperture represents that area which is normally occupied by the small membranous interventricular septum, the membranous atrioventricular septum and the junctional region of the atrial and ventricular septa. The aperture measures 3 cm. in an anteroposterior direction, and 1.4 cm. from the line of attachment of the atrioventricular valves to the free margin of the septum.

Both sides of the septum show abundant trabeculae, many of which are exceedingly fine. If the spaces between the trabeculae are carefully probed with a fine, blunt instrument, at least half a

MALFORMATIONS OF THE HEART INCLUDING TWO CASES
WITH COMMON ATRIOVENTRICULAR CANAL AND SEPTUM
DEFECTS AND ONE WITH DEFECT OF THE ATRIAL SEPTUM
(*COR TRILOCULARE BIVENTRICULOSUM*) *

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I. COMMON ATRIOVENTRICULAR CANAL AND SEPTUM DEFECTS

Although absence of the membranous part of the interventricular septum is a rather common heart anomaly, absence of the entire membranous septum combined with a common atrioventricular canal and an aperture in the lower atrial septum is quite rare. It represents a definite type of cardiac malformation, six cases of which are to be found described in the literature. In addition Keith¹ states that he has seen fourteen others and cites two described by Griffith, so that altogether we have found descriptions of, or reference to, twenty-two such cases. As the literature has not been exhaustively studied, some cases may have been overlooked, especially from European literature. However, recent discussion of the subject by Mönckeberg² seems to preclude this possibility.

CASE I

Clinical Report: The patient was a girl, one year old, a Mongolian idiot, who died in the Children's Hospital (No. 1384) with symptoms of cardiac insufficiency. Clinically, signs of an enlarged heart, a systolic murmur and gallop rhythm, led to the diagnosis of a congenital heart lesion. The previous history and family history of the case are irrelevant. The Wassermann reaction was negative. The necropsy took place eight hours after death.

Anatomic Diagnoses: (1) Defect of the atrial septum, with numerous perforations of the same, and patent foramen ovale; defect of the upper part of the ventricular septum; persistence of a common atrioventricular ostium, with abnormal development of the atrioventricular valves.

* Received for publication June 24, 1927.

connecting cusps in the normal heart. It is attached to the upper part of the interventricular septum anterior to the aperture by one of the several groups of chordae which comprise the anterior medial papillary muscle.

The anterior and lateral cusps of the tricuspid are attached as follows: the anterior, to the anterior medial papillary muscle and to the large anterior muscle; the lateral, which corresponds to the posterior cusp of the normal heart, to the large anterior and the posterior medial muscles. This is presumably normal. The lateral cusp, however, is poorly developed in that portion which lies against the posterior wall of the ventricle, being closely applied to the wall like the papillary muscle to which it is attached.

Lying against, and closely applied to the posterior half of the interventricular septum in the right ventricle, there is a cusp corresponding in size and position to the posterior half of the medial leaflet of the tricuspid. It arises from the upper posterior portion of the interventricular septum, and is attached in two places. The attachment to the posterior papillary muscle consists of only one or two chordae; the attachment to the septum is by several chordae lying beneath the cusp and by two long chordae stretching obliquely forward to be attached to the anterior half of the septum.

There remains, finally, a rudimentary cusp, the finger-like process referred to above. It is a fibrous mass, measuring 7 mm. in length and about 3 mm. in diameter. It projects freely into the aperture and is continuous over the posterior margin of the ventricular septum with the medial leaflet of the tricuspid. From its under surface there arise chordae which are attached, not directly to the septum, but to a narrow membrane, 7 mm. long and 3 mm. high, which in turn is attached to the upper margin of the interventricular septum in its posterior half.

The atrial septum shows conspicuous malformations. It consists almost entirely of a sheet of fibrous tissue, corresponding to the valvula foraminis ovalis of the normal heart. It is perforated all around its periphery by numerous foramina ranging in diameter from 1 to 5 mm. The muscular portion of the atrial septum (limbus) is limited to the upper anterior region of the septum and passes downward to become continuous with the musculature of the anterior wall of the atrium. The limbus does not quite overlap the upper anterior curved margin of the valvula foraminis ovalis and

dozen points of communication between the two ventricles may be found in different parts of the septum.

The common atrioventricular canal is surrounded by valve cusps which are better developed on the anterior than on the posterior side. They arise from the aorta and from the fibrous junction between atria and ventricles and are attached below to papillary muscles and to the anterior and inferior boundaries of the inter-ventricular aperture.

The anterior papillary muscle in the left ventricle is well developed and the posterior is absent. In the right ventricle all three papillary muscles, the large anterior, the anterior medial and the posterior medial, are well developed. The large anterior papillary muscle lies somewhat closer to the septum and has a rather more extensive attachment to it than is usual. The anterior medial papillary muscle, which consists of four or five groups of chordae, springs from the upper, anterior portion of the interventricular septum, or, dorsal wall of the conus, just anterior to the interventricular aperture. The two muscles comprising the posterior medial group do not stand away from the heart wall but resemble more closely the trabeculae carneae.

In the left ventricle a large cusp corresponding in position to the aortic cusp of the mitral valve is conspicuous. When viewed from the aortic side it is found to arise from the base of the aorta below the adjacent halves of the left and posterior semilunar cusps. It is attached to the anterior papillary muscle and to the anterior boundary of the interventricular aperture by chordae tendineae, and to the region between the right and posterior semilunar aortic cusps by a small fibrous sheet. The chordae which pass to the anterior margin of the aperture appear as if the membranous atrioventricular septum of a normal heart were shredded into chordae tendineae. The sheet of fibrous tissue which occupies the region between the right and posterior aortic cusps corresponds to the extreme upper portion of the normal, membranous, interventricular septum.

Laterally, the aortic cusp of the mitral is continuous with a small cusp which arises from the annulus fibrosus and which is attached to the anterior papillary muscle. Medially the large aortic cusp becomes continuous, by means of a strand of valve tissue, with a cusp in the right ventricle comparable in position to the anterior leaflet of the tricuspid. The strand is similar to those often seen

the atrial canal, as a prerequisite for the fusion of the endocardial cushions. They form, according to this author, a sort of bridge across which the cushions may travel toward each other. In cases of patent foramen ovale I (primary interatrial foramen) with normal valves, the ventricular septum alone performs this function. He attributes the persistence of the primitive undivided atrial canal to a primary growth failure of both septa. On the other hand, Mall, in describing the closure of foramen ovale I, assigns more extensive growth to the cushions and less to the atrial septum; indeed, according to him, the obliteration of this foramen is due to the continued growth and fusion of those parts of the cushions which project into it. In an embryo of 9 mm. he describes the fused cushions as a "cubical plug which blocks the center of the atrial canal"; he does not specifically state that the foramen ovale I in this embryo is patent, but at 11 mm. he states: "The complete union of the two cushions has obliterated the foramen ovale I." Whether fusion of cushions in the atrial canal antedates obliteration of the foramen, or is coincident with it, is not specifically stated, but certainly, according to Mall, the fusion of the cushions with the atrial septum, as well as obliteration of foramen ovale I, is due to growth on the part of the cushions, and the original atrial septum never extends down to the level of the atrial canal.

It seems to be generally accepted that the definitive atrial septum is formed of three elements, the septum primum, septum secundum and the lower portion of the left sinus valve (Tandler). After septum I is well developed and perforated to form foramen ovale II, septum II appears as a feeble elevation at the right of septum I, extending downward from the postero-superior wall of the atrium. Septum II, continuing to increase in height, extends forward along the superior wall of the atrium and then downward along its anterior wall, and finally backward to meet the forward projecting left valve of the sinus venosus. The union of these two forms the limbus. Mall, who does not describe the formation of the atrial septum, does not mention the septum secundum. To Mall, all the elevations participating in the division of the primitive atrium, atrial canal, and bulbus are but parts of the subendocardial tissue which he describes in considerable histologic detail; they are not sharply separated from each other. Thus he describes at 7, 8, and 9 mm., both before and at the time of the fusion of the endocardial

the muscular part is not fused to any extent with the fibrous septum. The atrial septum as a whole is not in the same plane as the ventricular septum. Its anterior attachment is in line with that of the ventricular septum, but its posterior line of attachment is displaced toward the right, so that the mouth of the coronary sinus which is located just to its right, lies, not above the medial cusp of the tricuspid, as in the normal heart, but above the posterior cusp. The posterior line of attachment of the atrial septum is 2 cm. to the right of that of the ventricular septum.

In the right atrium neither the valve of the coronary sinus nor the valve of the inferior vena cava is to be found. An extremely delicate fibrous cord, attached to the atrial wall just below and lateral to the mouth of the sinus, and above to the superior wall between the two venae cavae, is seen hanging free in the atrium. This may possibly represent the remains of an undeveloped right sinus valve.

INTERPRETATION

From our knowledge of the development of the heart, there can be no doubt that the malformation described depends upon two departures from the normal developmental processes: failure of fusion of the endocardial cushions, and persistence of the interventricular foramen. However, embryologists are not entirely agreed as to certain details in the normal course of these processes, so that before attempting to explain the anomalous features of this heart on the basis of these disturbances, the subject must be discussed in some detail. What seems fairly certain is that these disturbances date back to the first two months of intra-uterine life, for according to Mall³ the development of the septa, ending with the closure of the interventricular foramen, is complete at an embryonal length of about 16 to 18 mm.; the embryo according to Arey⁴ attains a crown-rump length of 17 mm. by the end of the seventh week.

On the subject of fusion of the endocardial cushions, and obliteration of the foramen ovale I, Mall differs from Tandler⁵ and from Mönckeberg in one fundamental respect. According to the last two authors the interatrial septum (primum) grows downward to meet the endocardial cushions and fuses with them; Mönckeberg particularly emphasizes the presence of the lower margin of the atrial septum and upper margin of the ventricular septum, at the level of

fibrous interatrial septum of the heart, separating the atria throughout at least three-quarters of their extent. Moreover, the upper free margin of the valvula foraminis ovalis is seen from the left side in its usual location at the upper anterior portion of the atrial septum. If, as Mall believes, the closure of foramen ovale I is due to the growth of the anterior and posterior cushions, the persistent interatrial aperture must be due to deficient growth of these cushions. The only features pointing to defective development of septum I, are the numerous small foramina perforating the fibrous septum. These might well be due to the fact that septum I was not reinforced all around its margin in the usual manner by septum II.

The obliquity of the atrial septum and the abnormal position of the coronary sinus, seem to be due to too great displacement of the opening of the sinus venosus. Early in embryonic development the opening of the sinus into the common atrium is made smaller by a fold coming in from the left side, and separating sinus from atrium (Tandler). In this way the sinus comes to open into the right side of the atrium. Septum I then develops just to the left of the mouth of the sinus. In this heart, the mouth of the sinus seems to have been pushed farther to the right than usual. The displacement of septum I may in turn have been partially responsible for the inability of the cushions to extend into and close foramen ovale I.

Regarding the interventricular septum, embryologists generally agree that coincident with the fusion of the atrial septum and cushions, the muscular interventricular septum increases in height and the bulbar septum grows down to meet it. During and after the fusion of these two septa, the interventricular foramen is reduced in size by continuous down-growth of the cushions. On the details of the above process, however, as well as in terminology, the various authors differ materially; they especially disagree on the exact mode of final closure of the interventricular foramen. It would lead us too far to go into the details of the controversy in which are included the opinions of Tandler, Mönckeberg, Sato, Born and Mall. Essentially the opinions held by these authors represent views varying more or less widely from the original description of His. It appears, however, that the diminution in size of the interventricular foramen is attributed to upward growth of the ventricular septum or down-growths from the cushions; its final closure to the bulbar septum, the cushions, or both.

cushions, prolongations of the cushions which extend upward "to the sinus venosus and are blended with the connective tissue above it." Again at 11 mm. he describes the fused mass (endocardial cushions) as extending up to the "atrial septum on its dorsal side and to the left valve of the opening into the sinus venosus." It is impossible to avoid the conclusion that the prolongations into the right atrium from this mass of connective tissue represent at least the lower part of Tandler's septum secundum. The attempt to apply Tandler's terms to these prolongations is made because it seems to establish an early continuity between the endocardial cushions, especially the posterior, on the one hand, and the septum secundum and sinus valves, especially the left, on the other. This connection is of interest in the anomalous heart under consideration.

In this heart the muscular portion of the atrial septum is defective. The lower limb of the limbus is absent. We may conclude that that portion of septum II which should have grown downward and curved backward, and there fused with the left valve of the sinus venosus, failed to develop in the usual manner. Furthermore, since the derivatives of the right sinus valve are also absent, a failure of development of both sinus valves seems to be indicated. It is possible, of course, that the valves of the coronary sinus and inferior vena cava were obliterated by the great dilatation of the right atrium, but more probably they were poorly developed, and hence left no trace in the atrium unless the fibrous cord mentioned above represents the right valve. If, as we have attempted to show, septum II of Tandler is either formed entirely, or increases in size, by the addition of those prolongations of Mall into the right atrium, then, failure of development of septum II and at least retarded development of the sinus valves, particularly the left, would in turn be dependent upon underdevelopment of the cushions, particularly the posterior. That in this heart the posterior cushion was deficient, will be shown later.

Another factor, however, may have contributed to the failure of fusion of the left valve of the sinus venosus with the endocardial cushions; namely, the obliquity of the atrial septum. Since the mouth of the coronary sinus lies so far to the right of the ventricular septum, this distance may have prevented the valve from fusing with the prolongation from the cushions.

Septum I apparently attained its usual growth, for it forms the

seem to be the only authors who have fully discussed this subject. Mall, who agrees with His, shows by the study of a graded series of human embryos that the medial cusp of the tricuspid is derived from the right ends of both cushions, and that the anterior medial papillary muscle is formed by the fusion of the septum aortopulmonale with the medial end of the right lateral cushion. It, therefore, marks the boundary between the anterior and right lateral cushions, and may be used in the interpretation of anomalous valve formation. Sato's theory of valve formation has usually been the accepted one, namely, that the right end of the anterior cushion takes part in the formation of the anterior cusp of the tricuspid.

It should be stated, in favor of the Mall theory, that Mall actually finds at one stage of development, the medial cusp of the tricuspid and the aortic cusp of the mitral hanging like curtains over the septum; this points toward the derivation of the medial cusp, like the aortic, from both endocardial cushions, and not as Sato believes, from the right end of the posterior only. Mall finds, also, in numerous embryos of less than 20 mm., that the two venous ostia are quite alike; each is bordered by a medial and lateral cusp, and each ventricle has a papillary muscle or set of muscles between the medial and lateral cusps. Finally, Mönckeberg describes in a trilocular heart, with single atrium but completely formed ventricular septum and atrioventricular cusps, the two medial cusps (aortic of the mitral, and medial of the tricuspid) continuous with each other over the top of the septum.

In this heart, the aortic cusp of the mitral is obviously derived from the left end of the anterior cushion, and is, therefore, the equivalent of that half of the normal aortic cusp which lies farthest from the septum. The small strand of tissue connecting the aortic cusp with the anterior of the tricuspid is, in the light of Mall's theory, derived from the right end of the anterior cushion; like the anterior end of the normal medial cusp, it is attached to the anterior medial papillary muscle of the right ventricle. The right end of the anterior cushion may have been more deficient in its growth potentialities than the left end. It may, however, have remained small secondarily, for all the cusps lacked the stimulus for development which would have been supplied had the cushions fused. Mall describes an attachment between the septum aortopulmonale and the right end of the anterior endocardial cushion similar to that be-

In the heart under consideration, the complete separation of the pulmonary artery and the aorta and the well developed upper anterior part of the ventricular septum speak for the normal development of the entire septum aortopulmonale. The numerous intertrabecular apertures discernible on probing may be regarded as evidence that the ventricular septum was somewhat underdeveloped. Mönckeberg regards these as remnants of the spongy trabeculae composing the septum. The large aperture is, however, not much larger than a normal membranous septum would be, and it seems quite probable that it was somewhat increased in size during dilatation of the heart. We conclude, therefore, that the ventricular septum was not greatly deficient in its growth.

Certain structures found in the place of the membranous septum and apparently derived from the anterior and posterior cushions, seem to indicate the part played by the cushions in the formation of the septum. For example, the chordae and fibrous leaf which attach the finger-like mass of endocardial tissue to the posterior margin of the aperture, may represent that process from the posterior cushion which, according to Mall, normally grows downward along the posterior margin of the foramen. Inasmuch as the chordae attaching the anterior cusp of the mitral valve to the anterior margin of the septum occupy the same position as the membranous septum of a normal heart, it would seem that they represent cushion material which normally goes into the formation of the membranous septum. In a recent report of a heart similar to this, Schleussing⁶ describes a small fibrous area lying "in the normal location of the pars membranacea" (between right and posterior semilunar aortic cusps?) and believes that this part of the septum membranaceum is formed by the bulbar septum. In our heart a similar small fibrous sheet is found, but it attaches the aortic cusp to the base of the right semilunar cusp, and is clearly similar to the chordae tendineae lying immediately below it. Our heart seems, therefore, to offer some evidence in favor of the theory advanced by Sato⁷ that the cushions are entirely responsible for the closure of the interventricular foramen.

Regarding the development of the atrioventricular valves, the original description of His seems, on the whole, to stand unchallenged. As to the exact part played by the endocardial cushions, both medial and lateral, in valve formation, His, Sato, and Mall

SUMMARY

The essential features of the heart described are as follows:

1. A large aperture of communication between the two sides of the heart, involving the lower atrial and the upper ventricular septa.
2. A fibrous interatrial septum, the posterior attachment of which, together with the coronary sinus, is displaced to the right.
3. A common atrioventricular canal bordered by five cusps — in the left ventricle, a small lateral and a large anterior, and in the right, a large anterior and small lateral and medial. The lateral corresponds to the posterior of the normal tricuspid valve.
4. A mass of endocardial tissue attached to the posterior margin of the interventricular septum, and projecting into the interventricular aperture.

It is obvious that the defects in this heart are the result of failure of fusion and imperfect development of the endocardial cushions, incomplete formation of septum II, and displacement of the mouth of the sinus venosus to the right. If Mall's description of the mode of closure of the foramen ovale I is accepted, the persistence of this foramen must be due to primary growth deficiency on the part of the cushions, rather than of septum I, as is commonly held. If the views of Mall regarding the relations of the lower portions of the left sinus valve are reconciled with those of Tandler, as we have attempted to do, it appears that imperfect formation of septum II may also be due to growth deficiency of the cushions. We have shown how the abnormal valves of this heart could be interpreted either in terms of Mall's or of Sato's descriptions of valve formation. Finally, since much of the site of the normal membranous interventricular septum is occupied by chordae tendineae, this heart seems to offer some evidence that the cushions normally play an important part in, if indeed they are not entirely responsible for, the closure of the interventricular foramen.

CASE 2

Clinical Report: The patient was a female child, aged four months, who died in the Children's Hospital (No. 2776). Physical examination showed Mongolian facies, malnutrition, cyanosis, a loud murmur over the precordium, and a short fifth digit. The Wassermann reaction was positive. The necropsy took place three and one-half hours after death.

tween the septum and lateral cushion. The only attachment acquired by the right end of the anterior cushion (to the septum aortopulmonale) tended, therefore, to draw it to the left, rather than out into the right ventricle and further hindered its increase in size.

As to the posterior cushion, the small medial cusp of the tricuspid was formed from its right end, and is, therefore, from Mall's standpoint, the equivalent of only the posterior half of the normal medial cusp. The posterior cushion failed to send a prolongation down into the left ventricle; consequently there is no cusp to correspond to the septal half of the aortic; in turn, no posterior papillary muscle was developed in the left ventricle. The posterior cushion material which should have gone into the aortic cusp is represented by the finger-like process. Marked underdevelopment of the posterior cushion is indicated by the character of its derivatives.

The right lateral cushion and the heart wall posterior to this cushion gave rise to the anterior and lateral (posterior) cusps of the tricuspid. The left lateral cushion gave rise to the small lateral cusp found in the left ventricle, which is the only representative in this heart of the normal posterior cusp of the mitral. The remainder of the posterior cusp, which should have been derived from the heart wall posterior to the lateral cushion by a process of undermining, was probably hindered in its development by the absence of the posterior papillary muscle.

The above interpretation of the anomalous valves, based on Mall's description of the embryologic development of the heart, seems to us most acceptable. Another interpretation, based on the theory of Sato, is, however, possible. According to his theory, the anterior cusp of the tricuspid is normally formed from the right end of the anterior cushion plus the major part of the right lateral cushion. In the light of this, the small strand of valve tissue which connects the aortic cusp of the mitral with the anterior of the tricuspid, represents, not the right end of the anterior cushion, but only that part of the cushion which lay above the interventricular septum. If this theory is correct, the anterior cushion was quite normal and the failure of fusion of the two cushions would have to be attributed almost entirely to growth failure of the posterior cushion.

lary muscle is rather small. The moderator band, extending from the base of the anterior papillary muscle to the ventricular septum, is unusually large in this heart, being thicker than the anterior papillary muscle itself, and short enough to hold the anterior and septal walls of the right ventricle in close apposition.

In the left ventricle there is a large cusp corresponding to the lateral half or two-thirds of the aortic cusp of the mitral. It arises from the lower margin of the adjacent halves of the left and posterior aortic cusps. Its lateral margin is attached to the anterior papillary muscle of the left ventricle. The upper part of its medial margin is attached by chordae tendineae to the anterior portion of the upper curved margin of the ventricular septum and to that muscular process which extends backward under the right aortic cusp. This attachment is made by chordae, which are not, however, completely separated from each other but are united into a sheet by a delicate web of endocardium stretching between them. From the lower part of the medial margin of the aortic cusp, chordae pass across the top of the septum to attach to the right surface of the septum.

The cusp just described is continuous by means of a narrow strand of valve tissue with the anterior cusp in the right ventricle. This strand is attached by chordae to the free margin of the septum in its upper anterior portion, and to the crista by means of some of the chordae (including the only fleshy member of the group) which we have called collectively, the anterior medial papillary muscle. The remainder of the chordae comprising this group attach the medial border of the anterior cusp of the right side to the crista supraventricularis; the lateral border of the same cusp is attached to the anterior papillary muscle. This cusp arises from the annulus fibrosus lateral to the conus. The lateral cusp in the right ventricle, which corresponds to the posterior of the normal heart, is attached by chordae both to the anterior and to the posterior medial papillary muscles.

The posterior cusp is well developed, in fact rather redundant, and is common to both ventricles, extending across the septum. In the right ventricle it is attached to the posterior medial papillary muscle, to the right surface of the septum and to the posterior part of the upper margin of the septum. Of those chordae which are attached to the septum some extend well forward to obtain attach-

Anatomic Diagnoses: (1) Defect of the atrial septum, with patent foramen ovale; defect of the upper part of the ventricular septum; common atrioventricular canal with abnormal atrioventricular valves. Patent ductus arteriosus.

(2) General hypertrophy and moderate dilatation of the heart especially of the right auricle. (Measurements in the fixed condition: apex to base, 4.3 cm.; transverse diameter, 3.8 cm.; circumference at base, 11.0 cm. Weight: 33 gm.)

(3) Mongolian facies.

(4) Purulent bronchitis and lobular pneumonia.

DESCRIPTION OF THE HEART

The muscular interventricular septum, which in its thickest portion measures 0.9 cm., has a concave upper margin which forms the lower boundary of the interventricular aperture. The aperture measures 1.5 cm. in an anteroposterior direction, and 0.7 cm. from the line of attachment of the atrioventricular valves to the free margin of the septum.

The upper anterior portion of the septum differs somewhat from that of the preceding heart. A muscular process from the anterior part of the septum extends backward and upward under the right aortic cusp to the posterior cusp. In the normal heart, as well as in the preceding, the area just under the point of junction of the right and posterior cusps is fibrous, forming in the normal heart, the uppermost part of the membranous septum; in this heart, this region is muscular.

Probing between the trabeculae of the ventricular septum reveals a number of points of communication between the ventricles.

The valve cusps surrounding the common atrioventricular canal are five in number — two anterior, which are continuous with each other through the aperture; a single posterior, common to both ventricles; and one lateral in each ventricle.

The papillary muscles of the left ventricle are normal, namely, an anterior and a posterior. In the right ventricle the posterior medial muscle may be considered normal. The anterior medial is represented, as in many normal hearts, by a group of chordae attached to the crista supraventricularis, or posterior wall of the conus; of this group, only one is fleshy. The anterior (large anterior) papil-

(i. e., probably that portion called swelling A by Tandler), as it grows down to meet the ventricular septum, divides into two limbs, one passing to the left, and the other to the right of the ventricular septum. The limb which passes to the right, carries the right branch of the atrioventricular bundle downward, and forms the moderator band. As was noted, the posterior cusp in this heart, obviously derived from the posterior cushion, is attached by chordae to a small papillary projection on the moderator band. For an explanation of this, three possibilities suggest themselves: (1) That Mönckeberg's theory of formation of the moderator band is incorrect; (2) that the muscle in question, in spite of its location, is not actually the moderator band; (3) that the posterior cushion, growing down into the right ventricle, obtained an accidental attachment to the moderator band. The latter possibility is not inconsistent with Mall's description of the formation of papillary muscles and chordae tendineae; nor do the chordae in question extend farther forward on the septum than chordae attaching the posterior half of the medial cusp of the tricuspid to the septum in many normal hearts.

BRIEF REVIEW OF SIMILAR CASES FROM LITERATURE

Preisz⁸ describes two cases, both in new-born children. In neither was the membranous atrial septum (valvula foraminis ovalis) as well developed as in these hearts; in both, the atrial canal was surrounded by four valve cusps, a large anterior and posterior, and two small laterals. In one case the anterior cusp was attached to the anterior limb of the interventricular septum; in the other, this attachment was absent.

Hart⁹ reports two cases, one aged two years, and the other ten months. Both showed large anterior and posterior atrioventricular valve cusps, whose chordae attached in the right ventricle. One of the cases, however, showed complete absence of the atrial septum. It is, therefore, a trilocular heart with ventricular septum defect, and does not strictly belong in the same class as the hearts under discussion. It has not been included in the total.

Mönckeberg describes one case in a child of nine months. The atrial septum was fibrous and perforated to form a foramen ovale II; its lower margin reached to a distance of 1 cm. from the atrioventricular canal; the septum secundum was entirely absent. The

ment to a rather prominent muscular projection from the moderator band. In the left ventricle the posterior cusp is attached to the left posterior papillary muscle. The lateral cusp in the left ventricle is narrow, and is attached to both anterior and posterior papillary muscles.

The atrial septum of this heart is somewhat better developed than that of the preceding. Though the disposition of the muscular portion is similar, ending below on the anterior wall of the right atrium, it comprises rather more of the atrial septum than in the preceding case. The fibrous portion (*valvula foraminis ovalis*) is thicker and not perforated. As in the preceding heart, its lower free margin forms the upper boundary of the interatrial aperture. The upper curved margin of the fibrous portion of the septum, falls just short of being overlapped by the muscular portion, thus forming a patent foramen ovale II. This septum is nearly in the same plane as the ventricular septum, its posterior attachment being displaced only very slightly to the left, instead of markedly to the right, as in the preceding case. Neither the valve of the coronary sinus nor of the inferior vena cava is present. As in Case I, there is a great discrepancy in size between the two atria and auricles (auricular appendices), the right being very much larger than the left. This difference is especially marked in the auricles.

INTERPRETATION

Since the heart in this case is in all essential features similar to the one previously described, the interpretation given for that heart will apply to this one. However, there are a few points of difference which must be discussed. The shifting of the mouth of the sinus venosus to the right, which in the preceding case, was assumed to explain the obliquity of the atrial septum, apparently does not occur in this heart, for the mouth of the coronary sinus lies directly above the right side of the ventricular septum. The derivatives of the posterior endocardial cushions are not so conspicuously underdeveloped as in the preceding. Both cushions are deficient, however, as compared to the normal.

The well developed muscle connecting the septal and anterior walls of the heart, which we have called the moderator band, presents difficulties. Mönckeberg believes that the septum aorticum

II. COMMON ATRIUM, COMPLETELY DIVIDED VENTRICLES, AND IMPERFECTLY FORMED TRICUSPID VALVE

This type of heart, with completely separated ventricles and common atrium, is called by Mönckeberg, *cor triloculare biventriculosum*.

CASE 3

Clinical Report: The heart is that of a male child, who died at the age of two months, in the Children's Hospital (No. 1971). The child was one of twins, born prematurely at the end of the seventh month of pregnancy. The only findings at necropsy, other than the abnormal heart, are general atrophy of the organs, hemorrhages in the pleura, lungs and capsules of the kidneys, and a marked slant of the palpebral fissures characteristic of Mongolism.

DESCRIPTION OF THE HEART

The heart is approximately normal in size and presents no abnormalities externally. When it is opened, the two atria are observed to be in wide communication. The atrial septum is represented only by a low muscular fold which extends from the left side of the coronary sinus to the lower margin of the defect just mentioned. The muscular fold extends forward for about two millimeters along the top of the membranous septum, forming the posterior part of the lower boundary of the aperture. The anterior part of the boundary is formed by the line of fusion of the membranous septum with the aortic cusp of the mitral valve.

The ventricular septum is completely formed. Its membranous portion is considerably larger than usual. The latter lies below the interatrial defect, and has a triangular process extending backward below the rudimentary atrial septum. On the right side, the membranous septum is not covered by the tricuspid valve as in the normal heart, because of malformation of this valve; a few small nodular thickenings appear on this surface. The top of the membranous septum is continuous with the medial third of the aortic cusp of the mitral, the two merging into each other without interruption.

Both cusps of the mitral and the papillary muscles of the left ventricle may be considered normal. The cusps of the tricuspid present certain malformations. Both they and the papillary muscles in the right ventricle are smaller than normal. With the exception of the posterior, the cusps are nodular and adherent to the septum or wall of the heart against which they lie. The posterior cusp is

common atrial canal was guarded by large anterior and posterior cusps (lateral cusps are not mentioned); and under the posterior aortic cusp was located a mass of endocardial tissue about the size of a grape seed. The midportion of the crest of the ventricular septum, forming the lower margin of the aperture, was free from valve tissue, as in our cases.

Schleussing⁶ reports two cases, one in a female Mongolian idiot of nine and one-half months, and another in a new-born child. The first, in which the atrial septum was similar to the first of our hearts, was complicated by some degree of transposition of the great vessels, and by the fact that the medial limb of the bulbar septum passed to the right of the ventricular septum. The valve cusps were five in number, a large anterior, continuous across the ventricular septum, two laterals and two posteriors. In the second case the atrial septum showed a large foramen ovale overlaid anteriorly and above on the right side by a perforated membranous fold. The cusps were four in number, a large anterior and posterior, and two laterals. He found in both cases a structure which he interpreted as the *pars membranacea*.

Keith¹ states that he has seen fourteen cases, all remarkably alike. He does not consider that the atrial septum was defective, though his figure shows a condition like that of the hearts described here. Unless the atria show a much larger aperture than in these cases he does not regard it as a persistent foramen ovale I. He states that failure of fusion of the endocardial cushions is always associated with some other grave defect, such as transposition of the great vessels or stenosis of the pulmonary artery; but in only two cases other than Keith's have defects of the great vessels been reported. In one of Preisz's cases the pulmonary valve had only two cusps; in one of Schleussing's cases there was partial transposition and narrowing of the aorta.

Keith also cites two cases similar to his, described by Griffith.

branous septum recalls the early fusion between an upward prolongation of the posterior cushion in the right atrium, and the left valve of the sinus venosus (Mall).

The cusps in the right ventricle will be interpreted first in the light of Mall's theory of valve formation. As noted previously, the anterior medial papillary muscle marks the boundary between the anterior and right lateral cushions. Examination of a series of normal hearts, has shown that both medial and anterior cusps are usually attached to the muscle and that it may consist of several parts, either muscular or tendinous. In the heart under discussion, it consists of a group of chordae attached to a fairly well developed papillary muscle, and a single chorda passing into the conus. The point between these two is taken as approximately the line of demarcation between the anterior and lateral cushions. The crumpled mass, therefore, represents the right end of the anterior cushion. Since this mass is distinctly separated from the medial cusp of the tricuspid, the latter, in this heart, seems to have been derived from the right end of the posterior cushion alone. The right ends of the cushions apparently failed to fuse.

Another interpretation, based upon the theory of valve formation as given by Sato, is possible. Sato holds that the normal medial cusp of the tricuspid is derived from the right end of the posterior cushion alone, and that the right end of the anterior cushion goes into the anterior cusp. The crumpled mass, if regarded as part of the medial cusp, must be a part of the posterior cushion, split off secondarily; if regarded as belonging to the anterior cusp, it is the right end of the anterior cushion. In the latter case, it must be assumed that the growth potentialities of the posterior cushion were insufficient to form a normal medial cusp.

In a recent study of malformations of the heart, Mönckeberg has no cases exactly similar to this one. He describes, however, one case of *cor triloculare biventriculosum*, in which there was no vestige of the atrial septum, and in which the apex was cleft.

SUMMARY

The heart just described presents, in addition to almost complete absence of the atrial septum, defects of the tricuspid valve, especially its medial cusp. The defect of the atrial septum is due to absence

quite free; it arises from the posterior and lateral portions of the atrioventricular junction, and sends numerous fine chordae tendineae to the posterior medial papillary muscle and to the adjacent portion of the septum. The medial cusp is deficient in its anterior one-third; it arises from the upper margin of the muscular portion of the interventricular septum. It is adherent to the septum and might be described as a patch of nodular and thickened endocardium on that portion of the septum. Its lower margin, however, is free, and from this margin chordae pass to the posterior medial papillary muscle and to the septum. It has also a single chorda passing to the lowest point of junction of the membranous septum with the muscular septum.

Between the membranous septum and the crista supraventricularis there is a crumpled mass of valve tissue from which two groups of chordae pass, one to the surface of the membranous septum itself and to the lowest point of junction of membranous with muscular septum, and the other to the lowest point of the crista. The latter group of chordae are gathered together and are attached to a single, fairly well developed papillary muscle which constitutes the major portion of the anterior medial muscle.

The crumpled mass just described is directly continuous with the anterior cusp of the tricuspid. This cusp is fairly smooth, but, like the medial, is closely adherent to the heart wall, excepting that portion which joins it to the crumpled mass. From this junctional piece, a single chorda passes across the crista and is attached within the conus to its posterior wall. This chorda and the single papillary muscle mentioned above, comprise the anterior medial papillary muscle. The chordae of the anterior cusp pass to the large anterior papillary muscle. The latter is poorly developed, consists of two trabeculae, and lies against the lateral (anterior, in the normal position of the heart) wall of the ventricle.

INTERPRETATION

That septum I was deficient or entirely absent in this heart seems clear. Because of its location medial to the mouth of the coronary sinus, the rudimentary atrial septum, which is muscular, seems to have been derived from the left valve of the sinus venosus. It corresponds to the lower and posterior part of the normal limbus of the fossa ovalis. Its continuity with the posterior part of the mem-

DESCRIPTION OF PLATES

PLATE 156

FIG. 1. Left atrium and ventricle of heart from Case 1. The finger-like process projecting into the aperture from the posterior heart wall is seen to the right of the aperture.

FIG. 2. Right atrium and ventricle of heart from Case 1. The finger-like process is here seen projecting into the aperture from the left.

or degeneration of the septum primum, and to absence of the septum secundum. The rudimentary atrial septum is interpreted as the left valve of the sinus venosus. The defective medial cusp of the tricuspid is interpreted as due either to failure of fusion of the right ends of the endocardial cushions (if Mall's description is accepted), or to deficient growth potentialities of the posterior cushions alone (according to the theory of Sato).

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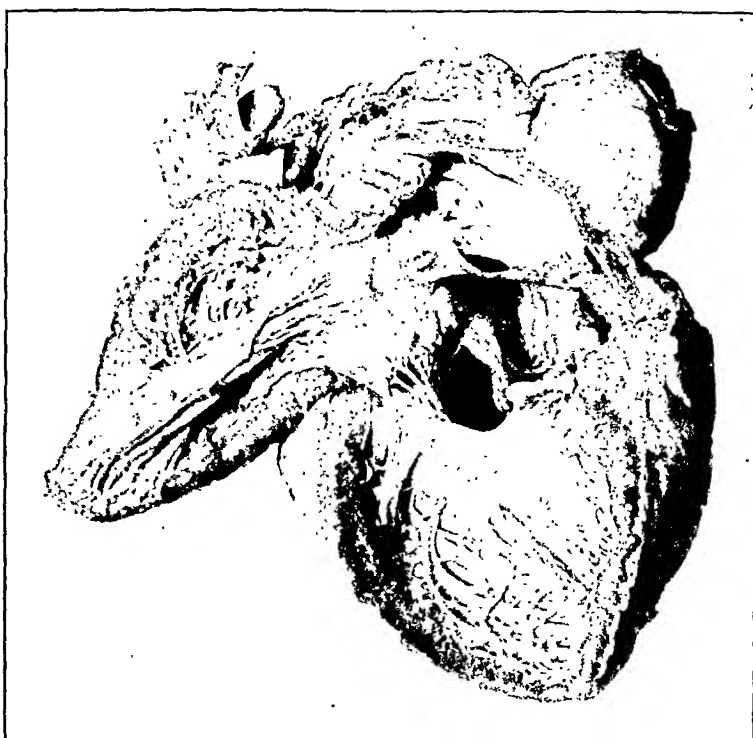
PLATE 157

FIG. 3. Region just below the aorta from the heart of Case 1. The attachment to the base of the right semilunar cusp is by a small sheet of fibrous tissue, and to the anterior margin of the aperture by chordae tendineae.

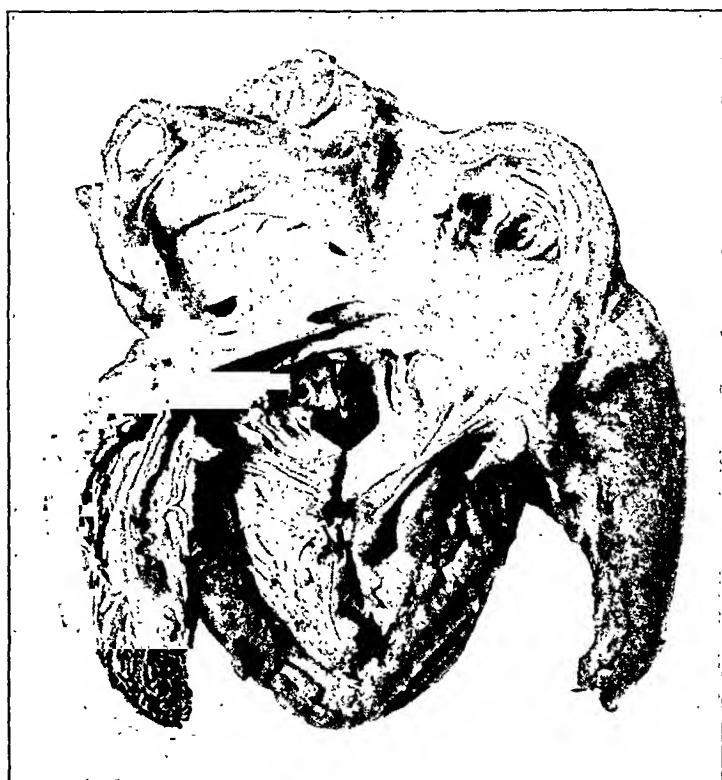
FIG. 4. Left atrium and ventricle of heart from Case 2.

FIG. 5. Right atrium and ventricle of heart from Case 2.

FIG. 6. Region just below the aorta from the heart of Case 2. The attachment to the muscular process that extends backward under the right semilunar cusp as well as to the upper part of the anterior margin of the aperture is by chordae tendineae, which are joined together by a delicate web of endocardial tissue. The lower chordae pass through the aperture to the right side of the top of the septum.



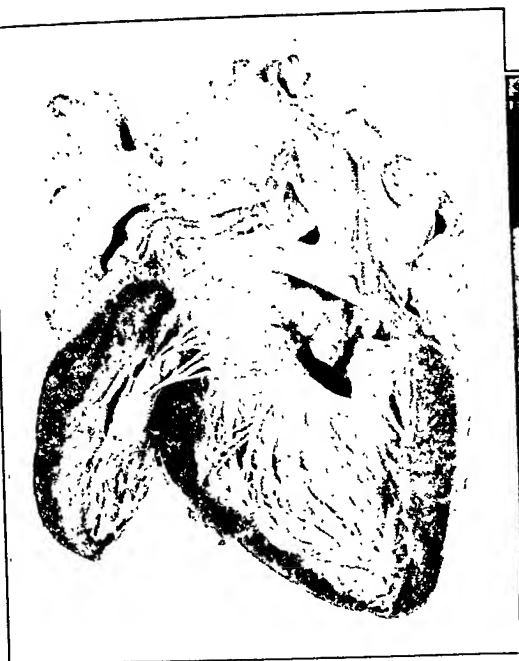
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2

PLATE 158

FIG. 7. Right atrium and ventricle of heart from Case 3, the anterior wall of the heart removed.



3



4



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6



parently of long standing, was noted. A "machinery" murmur was heard over the sternum. Respirations were slow and labored. The pupils were active and equal, and there was bleeding into the posterior pharynx. The patient did not regain consciousness and died four hours after admission. A history of previous cardiac symptoms was not obtained.

Necropsy: The examination was performed eleven hours after death. Only the findings bearing on the subject are here included. The body is fairly well developed and nourished and weighs 47.7 kilograms. The finger nails have a decided double curvature, but there is no clubbing of the phalanges. As the chest plate is removed it is noted that the heart's transverse diameter is unusually great and that the diameter from base to apex is relatively short. No pulmonary artery is seen *in situ*, and so the heart and lungs, together with the aorta as far as the bifurcation of the common iliac arteries are removed *en masse*.

The heart is large and very firm, and is estimated to weigh over 500 gm. The apex is broad and not very well defined. The epicardium is smooth and glistening but for a gray elevated plaque 3 cm. in diameter on the anterior surface near the base. A moderate amount of subepicardial fat is present along the course of the coronary arteries. The auricular appendages nearly surround the common truncus arteriosus (Fig. I, T. A.), which arises from the left side of the base of the heart. No evidence whatever is present of a pulmonary artery or of a ductus arteriosus. The ascending portion of the truncus is wide, and a short distance beyond its junction with the transverse portion gives rise to the right innominate, left common carotid and left subclavian arteries in the normal manner. Just beyond this latter vessel the truncus narrowed perceptibly, and here, from the right lower surface of the arch of the truncus, arises a vessel (Fig. I, 1) which courses beneath the arch toward the left and enters the upper lobe of the left lung. At its origin from the truncus this vessel measures 6 mm. in diameter. One and one-half centimeters beyond this, from the right side of the truncus, another vessel (Fig. I, 2) arises, measuring 8 mm. in diameter at its origin. This vessel passes transversely in front of the trachea, just above the bifurcation, to enter the upper lobe of the right lung. This vessel bifurcates just before it enters the lung. One centimeter beyond this

A CONGENITAL ANOMALY OF THE HEART: TRUNCUS ARTERIOSUS COMMUNIS*

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In view of the infrequent occurrence of the congenital anomaly of the heart, truncus arteriosus communis, it seems desirable to report a case of this kind which occurred during the past year on the Pathology service of the New Haven Hospital. Moreover, several unusual features make this case particularly interesting; namely, the age of the patient, the unusual blood supply to the lungs and the apparent lack of previous cardiac symptoms. No complete review of the literature is attempted, for Dr. Abbott has done this in a recent article¹ in which a summary of the literature and other sources of study reveal but twenty-three cases of this anomaly. The case history given below is that of the oldest patient presenting a truncus arteriosus communis to come to necropsy. Vierordt reported one dying at sixteen and another at nineteen years of age. Crisp's patient lived to twelve years. The other patients in this series died in early childhood or infancy.

The embryologic factors responsible for cardiac malformations are discussed in detail by Abbott and Shanly,² and, therefore, will not be repeated here. The arteries supplying the lungs in this case, one to each lobe, appear to be derivatives of the branchial arches of the fetus.

REPORT OF CASE

History: A fairly well developed and well nourished black male, aged 25 years, was brought to the New Haven Hospital in an unconscious condition as the result of an automobile accident. On admission the patient had a contusion and laceration over the left eye. The pulse was 68 per minute and the blood pressure 140 systolic and 74 diastolic. A regular irregularity of the heart, ap-

* Received for publication July 5, 1927.

is the remains of the pars membranacea. This membranous structure is inserted above between the anterior and the right posterior cusps of the common truncus. Below it is attached to the medial cusp of the tricuspid valve by a structure resembling a chorda tendinea. Its vertical position serves to partially narrow the interventricular defect. This ventricle lacks a well marked conus arteriosus, the space between the anterior heart wall, the anterior cusp of the tricuspid valve and the right side of the remains of the pars membranacea being quite shallow.

The large truncus arteriosus arises chiefly from the left ventricle, only about one-fourth of the lumen over-riding the interventricular defect. The semilunar cusps guarding the orifice of the truncus are three in number and very large, and the sinuses of Valsalva are all very deep, readily allowing the insertion of the end of the thumb. This valve measures 8 cm. in circumference. The sinus of Valsalva behind the anterior cusp is deeply funnel-shaped. The right and left coronaries arise rather high up in the anterior and in the left posterior sinuses, respectively. No evidence whatever of the septum aortopulmonale is present, and a slight puckering of the wall of the truncus 4 cm. above the truncal valve is the only evidence of a probable ductus Botalli. The base of the truncus has a few irregularly shaped yellow arteriosclerotic subintimal plaques.

The mitral valve is delicate and velamentous and the chordae tendineae insert well beyond the free edge. From the above description of the heart, it is seen that the venous blood of the right ventricle mixed with the arterial blood of the left ventricle at each ventricular systole, for the only pathway of escape for the blood in the right ventricle is through the interventricular defect into the common truncus, which received simultaneously the blood from the left ventricle.

The following anatomic diagnoses are made after a complete necropsy:

Primary: Contusions and lacerations of the face and body; extradural hemorrhage (following rupture of middle meningeal artery).

Subsidiary: Common truncus arteriosus persistens and interventricular septal defect; apical pulmonary scar; pleural and peritoneal adhesions.

vessel, and arising from the same side of the truncus, a small artery (Fig. II, 3) 2 mm. in diameter, passes transversely behind the lower portion of the trachea to enter the middle lobe of the right lung. Here also, from the under surface of the arch of the truncus, arises an artery (Fig. II, 4) 4 mm. in diameter passing obliquely downward to supply the lower lobe of the right lung. One centimeter beyond this last vessel, and from the left side of the beginning of the descending portion of the truncus, arises an artery (Fig. II, 5) 12 mm. in diameter, which passes horizontally toward the left to enter the lower lobe of the left lung. Just before it enters the lung this vessel also bifurcates. At this level the truncus, which follows the normal course of the thoracic and abdominal aortae and gives rise to the arteries usually derived from these vessels, measures 4.5 cm. in circumference.

The cavity of the right atrium is approximately three times the size of the left. The foramen ovale is closed. The valves of the inferior vena cava and coronary sinus are well formed. Two left pulmonary veins open by a short common trunk into the very small left auricle and two similar veins from the right lung also unite a short distance from the lung to enter this auricle.

The left ventricular wall measures 15 mm. in thickness, and the right wall at a corresponding level measures 12 mm. The interventricular septum averages 20 mm. in thickness. The capacity of the left ventricle is at least three times that of the right. The endocardium of all four chambers is everywhere smooth and glistening. The papillary muscles of both ventricles are greatly hypertrophied and the columnae carnae stand out as thick cords from the ventricular walls. The tricuspid valve is delicate and velamentous and the chordae tendineae insert well beyond the free edge of the valve. Two chordae arise from the muscular interventricular septum below and in front of the septal defect and insert into adjacent parts of the anterior and median cusps of this valve. This interventricular defect measures 1.5 cm. in diameter and readily permits the insertion of one finger. It is situated between the upper border of the ventricular septum and the lower surface of the semilunar valves of the common truncus. Its anterior, posterior and lower limits are formed by the anterior and posterior walls and the muscular septum respectively. Above, it is bounded by a valve-like structure which

DESCRIPTION OF PLATES

PLATE 159

FIG. 1. Anterior view of heart showing common truncus and origin of pulmonary arteries to upper lobes of lungs.

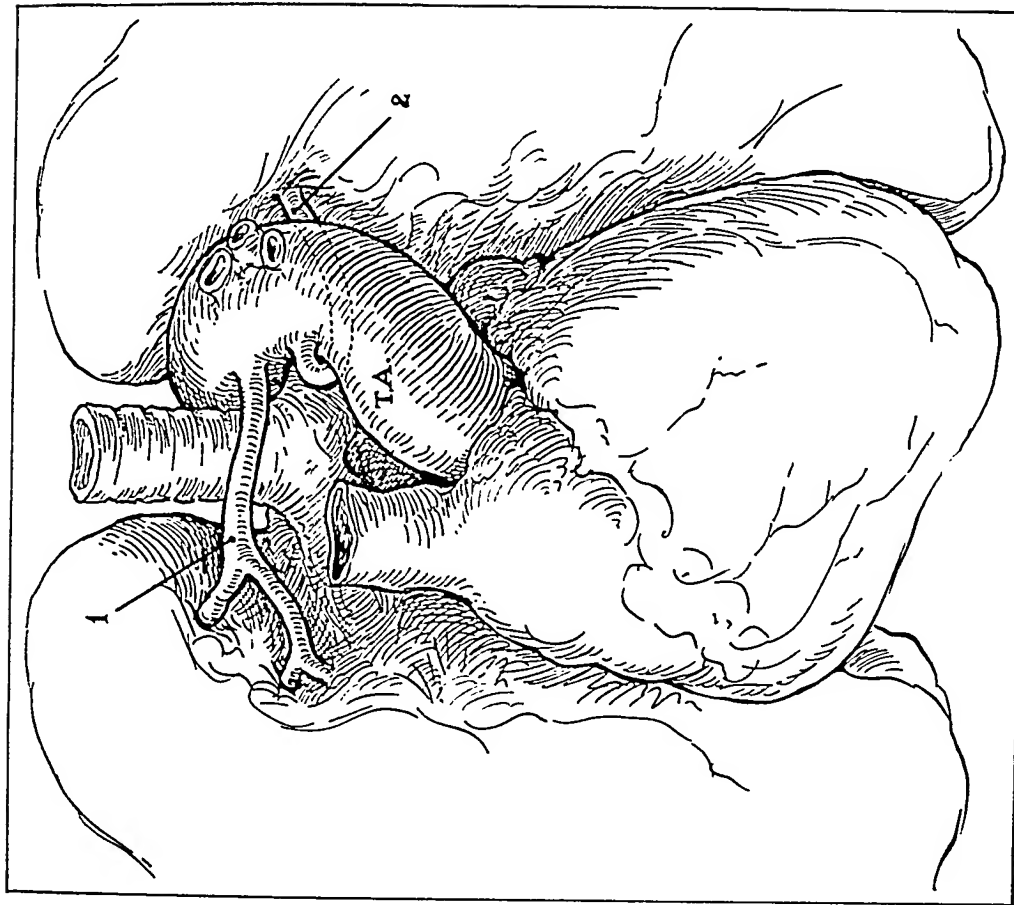
FIG. 2. Posterior view of heart showing common truncus and origin of pulmonary arteries to lower lobes of lungs.

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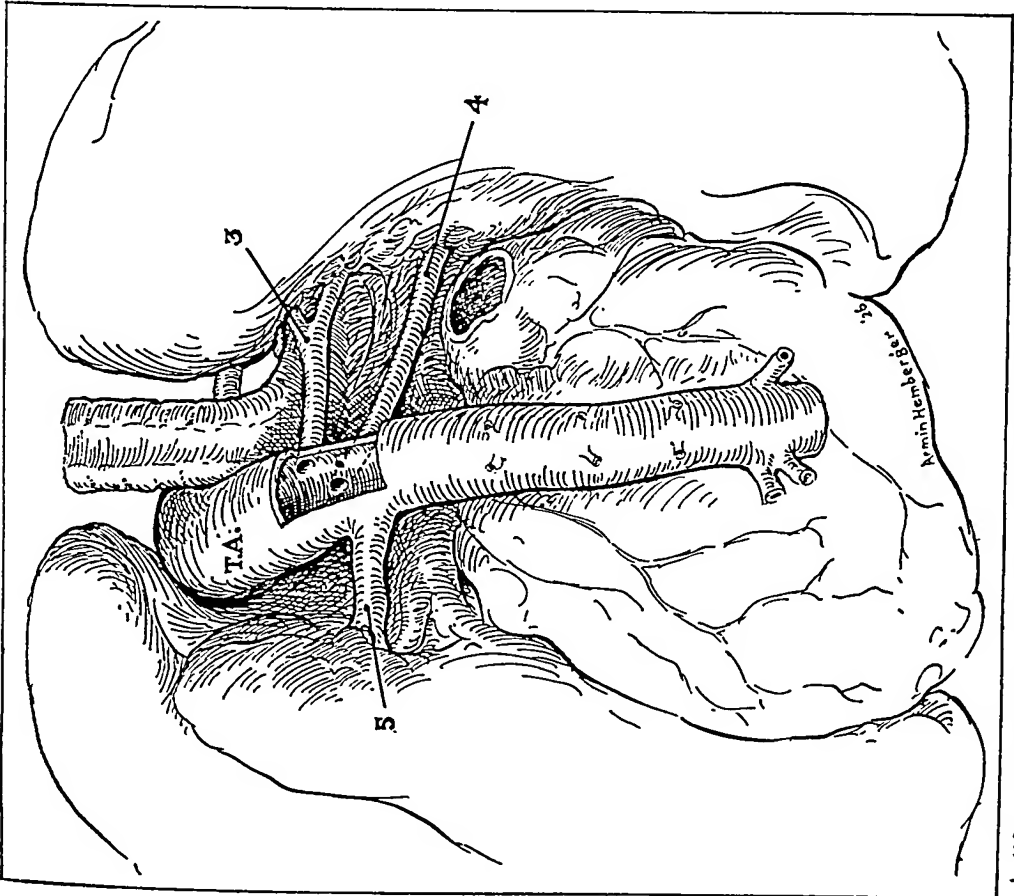
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PLATE 160

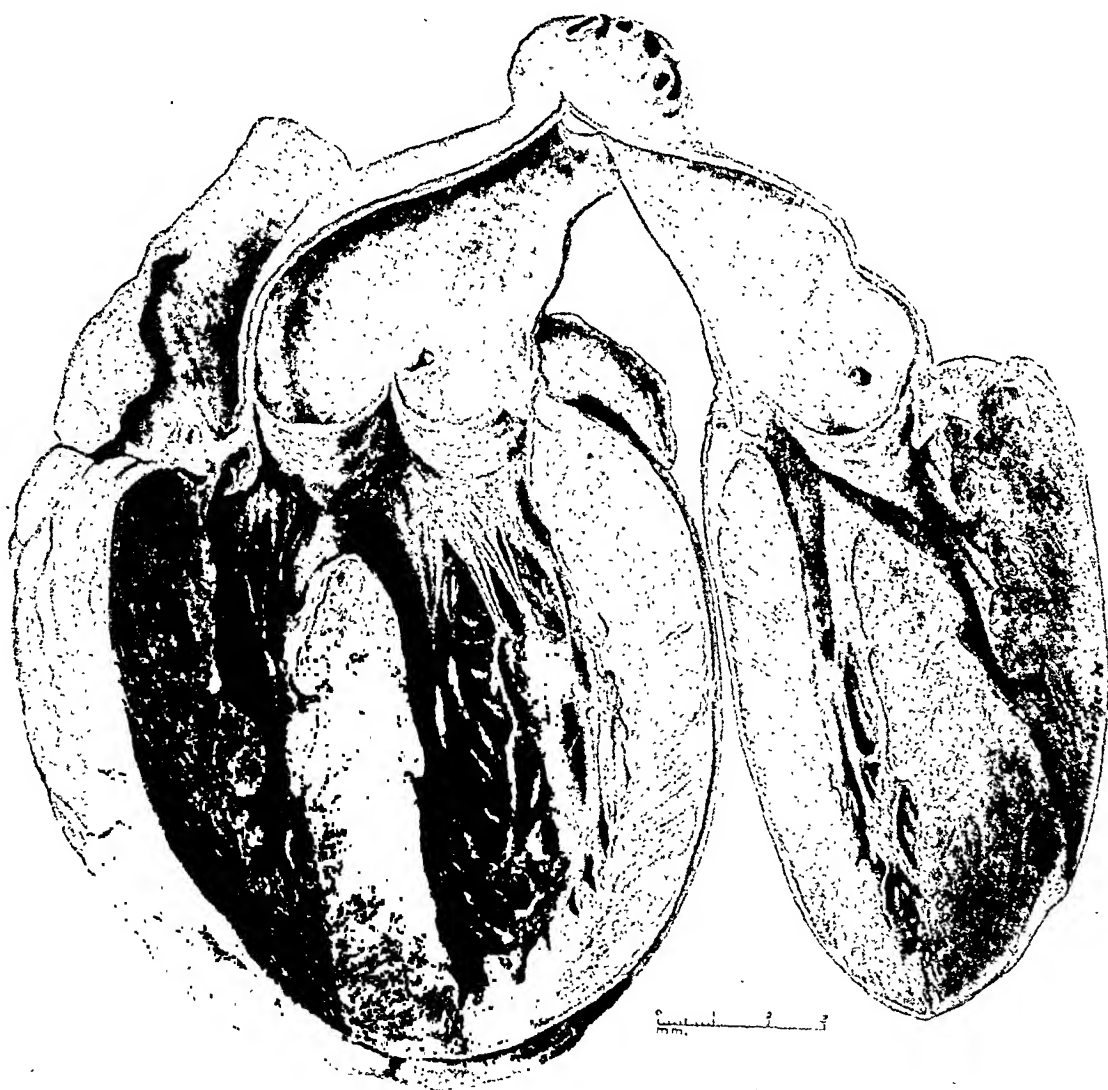
FIG. 3. Longitudinal section of heart showing common truncus arteriosus and interventricular septal defect.



A.1190 ANTERIOR VIEW OF HEART SHOWING COMMON TRUNCUS AND ORIGIN OF PULMONARY ARTERIES TO UPPER LOBES OF LUNGS. 1



A 1190 POSTERIOR VIEW OF HEART SHOWING COMMON TRUNCUS AND ORIGIN OF PULMONARY ARTERIES TO LOWER LOBES OF LUNGS 2



A. 1190 LONGITUDINAL SECTION OF HEART SHOWING
COMMON TRUNCUS ARTERIOSUS AND
INTERVENTRICULAR SEPTAL DEFECT. □

age, entered the hospital after two weeks of headache, vomiting and joint pain. The joints of the ankles and fingers were swollen and tender. Systolic and presystolic murmurs were present at the apex. The condition of the patient grew steadily worse and he died in coma at the end of a month. There was no evidence of meningitis. Meningococci grew in pure culture from the blood. The necropsy showed an adhesive pericarditis, and a thickened mitral valve with a soft, cauliflower vegetation on the anterior leaflet. Some thickening of the aortic valve and infarcts of the spleen and kidney were found. Smears and sections of the vegetation showed Gram-negative intracellular diplococci. The bacteriologic examination in this case was extremely thorough. The organism corresponded to the meningococcus in morphology and cultural characteristics. It was pathogenic for animals, fixed complement and was agglutinated by antimeningococcus serum.

In 1912 Finley and Rhea ² recorded a case in a man, 47 years of age, who entered the hospital after three weeks of joint and precordial pain, chills and cough. There was a soft, systolic murmur at the apex, an enlarged liver, a hemorrhagic eruption of the skin and a painful and swollen finger. Meningococci were grown from the blood and spinal fluid. At the necropsy a narrow row of irregular gray-yellow, friable vegetations were found on the mitral and aortic valves. There were infarcts of the spleen and kidneys. Meningococci were obtained on culture of both vegetations and infarcts.

Mackarell ⁴ in 1915 recorded two cases. The first patient showed a systolic murmur at the apex, and meningococci in blood culture. Death ensued after an illness lasting twelve weeks. The second patient was ill for sixteen weeks with meningeal signs; and a pure growth of meningococcus was obtained from the spinal fluid. The mitral valve in both of these cases at necropsy showed large, granular, friable vegetations in which meningococci were demonstrated.

Fairley and Stewart ³ (quoted by Worster-Drought and Kennedy ⁵) in 1916 also reported two cases. One showed fresh granular vegetations of good size on sclerosed mitral and aortic valves. The second case had fresh vegetations on two cusps of the aortic valve. In both of these cases cultures of the vegetations showed a pure growth of meningococcus.

Worster-Drought and Kennedy ⁵ in 1919 recorded a case of endocarditis in a man, 37 years of age, who had a definite history of

VEGETATIVE ENDOCARDITIS DUE TO THE MENINGOCOCCUS *

WITH A CASE REPORT

C. P. RHODES, M.D.

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Cases of vegetative and ulcerative endocarditis due to the meningococcus are unusual enough to justify placing them on record. In a fairly careful survey of the literature, reports of only eleven cases were found. In some of these cases the accuracy of the diagnosis is questionable because the details of the bacteriologic examinations are not given.

The first case was reported by Warfield and Walker ⁹ in 1903. The patient entered the hospital after two weeks of headache, cough, chills and delirium. There was a loud systolic murmur at the apex. At no time were signs of meningitis observed. A pure culture of meningococcus was obtained from the blood. At necropsy, acute ulcerative endocarditis of the mitral valve, mitral stenosis, and infarction of the spleen and kidneys were found. Meningococci in pure culture were obtained from the vegetation.

Weichselbaum and Ghon ⁸ in 1905 reported a case of typical epidemic cerebrospinal meningitis in a child 9 years of age who died in the fifth week of the disease. The necropsy showed meningitis, pericarditis and vegetative endocarditis of the mitral valve. Meningococci were cultured from the vegetation.

Westenhoeffer ⁷ in 1906 reported two cases of endocarditis associated with meningitis. The first, a child 1 year old, exhibited a typical example of epidemic cerebrospinal meningitis with death at the end of a month. At necropsy, meningitis and vegetative endocarditis of the mitral valve were found.

The second case was that of a woman 21 years of age who died after a five-day illness during which signs of meningitis were observed. The necropsy again showed meningitis and vegetative mitral endocarditis.

Cecil and Soper ^{1,6} in 1911 made very careful observations on a case at the Presbyterian Hospital. The patient, a man 31 years of

* Received for publication August 18, 1927.

Heart: The weight is 490 gm. It is somewhat larger than normal. The epicardium is smooth and glistening. The myocardium is firm and a dark brick-red color. There is no gross evidence of fibrosis. Projecting into the right auricle just below the fossa ovale is a soft, smooth, purple-red, friable mass, 1.5 cm. in diameter, which involves the medial cusp of the tricuspid valve. This vegetation fills a ragged perforation about 1.5 cm. in diameter which extends through the interventricular septum. On the left side of the heart it forms an irregular hole just below the medial cusp of the aortic valve and includes the base of the valve to the line of closure. The edges of the perforation are covered with brown, granular material which is easily broken off. Smears of the vegetation show a mass of fibrin, polymorphonuclear cells, and Gram-negative diplococci. The endocardium and valves are otherwise entirely negative. The coronaries are not remarkable.

Lungs: The upper portions are soft, spongy and gray. The lower portions are pink, somewhat firmer and crepitant. Multiple sections show no gross abnormality.

Spleen: The weight is 320 gm., and the size somewhat greater than normal. The surface is slightly wrinkled and dark purple in color. The cut surface is dark red with distinct gray markings. The consistence is firm and a small amount of pulp scrapes away.

Gastro-Intestinal Tract: Negative.

Pancreas: Negative.

Liver: The weight is 2360 gm., and the organ is somewhat larger than normal. The surface is smooth and reddish brown. The consistence is firm. Multiple sections reveal no gross pathology. The gall bladder and ducts are negative.

Kidneys: The combined weight is 420 gm. The left kidney contains several sharply circumscribed, gray to yellow areas which are pyramidal in shape with base toward the surface and the apex toward the pelvis. In these regions the normal kidney markings are obliterated. The surface of the kidney over these areas is somewhat raised, granular and adherent to the capsule. In the right kidney there are two similar lesions.

The *adrenals, bladder, genitalia* and *aorta* are negative.

rheumatic fever. There were signs of sepsis without evidence of meningitis and he died after an illness lasting five weeks. The heart was enlarged and both systolic and diastolic murmurs were heard over the aortic area. Meningococci were grown from the blood. At necropsy a perforation of the basal portion of one aortic cusp was found which left the free edge unaffected. Projecting from the ulcerated area was a massive, tough, fibrinous vegetation, which contained meningococci.

The following is a summary of a case seen in this hospital:

Report of Case: Patient, H. L., a male negro laborer, 21 years of age, entered the Boston City Hospital Jan. 23, 1927, complaining of chills and dyspnea. The family and the past history are negative.

Present Illness: Two weeks before coming to the hospital the patient noticed marked dyspnea and palpitation, with a slight cough. There were several severe chills and the patient felt feverish up to the time of admission. He also suffered several attacks of very intense precordial pain radiating down both arms.

Physical Examination: The patient was a well nourished man, rational and quiet, exhibiting moderate dyspnea and marked cyanosis. The heart was slightly enlarged to the left. A blowing diastolic murmur was heard over the aortic area. At the apex a short presystolic and a loud blowing systolic murmur transmitted to the axilla were present. A fine thrill was felt at the apex. The rate was 140 a minute, the rhythm regular, and the sounds of fair quality. The pulse was Corrigan in character. Pistol-shot sounds were heard in the cubital fossae, groins and popliteal spaces. A capillary pulse was noted. The lungs showed dullness and rales at both bases. There were no signs of meningitis. The temperature was 101° F and the respirations 34 a minute. The blood pressure was 140 systolic and zero diastolic. The leucocyte count was 15,000. The urine showed a large trace of albumen with many red and white blood cells and brown granular casts in the sediment. The clinical diagnosis was acute endocarditis with aortic regurgitation.

Course of Disease: There was little change up to the time of death. The temperature subsided to 99° F and the rate of respirations increased somewhat. On the third day after admission he suddenly collapsed and died.

NECROPSY REPORT

Necropsy: Performed Jan. 26, 1927, one hour postmortem. Enlarged axillary and enlarged and indurated inguinal nodes are felt.

The liver edge is 14 cm. below the xiphoid cartilage and 8 cm. below the costal margin in the right mid-clavicular line. There are a few slightly enlarged mesenteric lymph nodes.

The right pleural cavity contains about 300 cc., and the left 100 cc. of straw-colored fluid. A few fine fibrous adhesions in the right pleural cavity are noted.

they stained with the dilute carbol fuchsin used as the counter-stain in Gram's method.

Agglutination: Suspensions of twenty-four hour cultures on hydrocele agar slants were made in salt solution and kept at 56° C for one hour. Mixtures of equal parts of the bacterial suspension with graded dilutions of a standard antimeningococcus agglutinating serum with a titer of 1:400 were made. The mixture was kept twenty-four hours at a temperature of 55° C. Agglutination was complete up to a dilution of 1:100 and partial to 1:400. Controls with salt solution in place of the bacteria and antipneumococcus serum in place of the antimeningococcus serum were negative. A culture of gonococci similarly treated was not agglutinated.

Precipitins: The organisms were grown for twenty-four hours on hydrocele agar slants. Each tube was then washed down with 10 cc. of sterile distilled water and shaken well; the tubes were pooled, and phenol was added to the mixture to give a concentration of 0.5 per cent. The flask was kept at room temperature for a week and shaken daily. The material was then centrifuged at high speed for twenty minutes and the water-clear supernatant fluid was pipetted off and used as antigen. Standard agglutinating serum for the meningococcus was used as the source of the antibodies. A good precipitin ring was formed with an antigen dilution up to 1:600. Horse serum in place of the antimeningococcus serum and salt solution in place of the antigen gave no precipitation. An antigen made in the same fashion from a culture of gonococci gave no precipitation.

Complement Fixation: An antigen made in the same way as that used for the detection of precipitins was used. Inactivated antimeningococcus serum was used as amboceptor: 0.05 cc. of antigen fixed complement down to the point where 0.0025 cc. or less of anti-serum was used. There was no fixation using horse serum in the place of the antimeningococcus serum.

SUMMARY

A case of vegetative and ulcerative endocarditis due to the meningococcus is reported and the literature reviewed.

The identity of the organism is proved culturally and serologically.

MICROSCOPIC REPORT

Heart: The epicardium is thickened and infiltrated with lymphocytes. Between the muscle fibers there are many endothelial leucocytes, polymorphonuclear neutrophiles, eosinophiles and lymphocytes. There are a few areas which show necrotic bundles of collagen fibers surrounded by endothelial leucocytes, strongly suggesting Aschoff bodies. In certain areas there is fairly extensive necrosis of muscle fibers with replacement by granulation tissue.

Sections through the vegetations show a mass of fibrin thickly infiltrated with polymorphonuclear and endothelial leucocytes. There are masses of Gram-negative diplococci present both within and outside of cells. These can be seen in sections stained with Mallory's eosin-methylene blue, Giemsa's method and MacCallum's modification of Goodpasture's stain.

Lung: The alveoli contain a few erythrocytes, rare polymorphonuclear cells and a few endothelial leucocytes.

Spleen: A number of areas are present, particularly marked in the germinal centers, where endothelial leucocytes are grouped with masses of fibrin and some polymorphonuclear cells. Around these clumps of cells there is a proliferation of fibroblasts. Scattered throughout the organ are focal collections of plasma cells.

Liver: In the region of the central veins a few liver cells that show the hyaline cytoplasm of early necrosis are seen. A slight lymphocytic infiltration is present in the portal areas.

Kidney: There are areas of infarction necrosis where the normal structure is completely obliterated. At the periphery of these areas masses of erythrocytes and polymorphonuclear cells are grouped.

Adrenal: Negative.

BACTERIOLOGY

Cultivation: Smears of a vegetation grown forty-eight hours on sheep blood-beef infusion agar plates showed an even, sparse growth of smooth, rounded, pearly-gray, non-hemolytic colonies. The best growth took place on hydrocele agar. No growth could be obtained in liquid media.

Morphology: Smears from the vegetation and from cultures showed a pure growth of Gram-negative, biscuit-shaped diplococci. They varied greatly in their size and in the intensity with which

DESCRIPTION OF PLATES

PLATE 161

FIG. 1. Photograph of the right side of the heart showing a large, rounded vegetation projecting from the inner surface of the auricle and the tricuspid valve. Under the vegetation there has been necrosis of the interventricular septum with perforation through to the aortic valve which is shown in Fig. 2.

Lesions of the myocardium resembling those seen in rheumatic fever were obtained.

I am indebted to Dr. F. B. Mallory and Miss Catherine G. Norton for the illustrations and to Miss Lillian M. Leavitt for technical assistance.

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PLATE 162

FIG. 2. Beneath the aortic valve is a perforation of the interventricular septum and ulceration of the valve cusps with formation of vegetations.



1

PLATE 163

FIG. 3. Photomicrograph of a section through vegetations composed chiefly of dead organisms. The dark edge is formed by masses of living cocci. $\times 50$.

FIG. 4. Section through a small vegetation in which suppuration has taken place. $\times 40$.

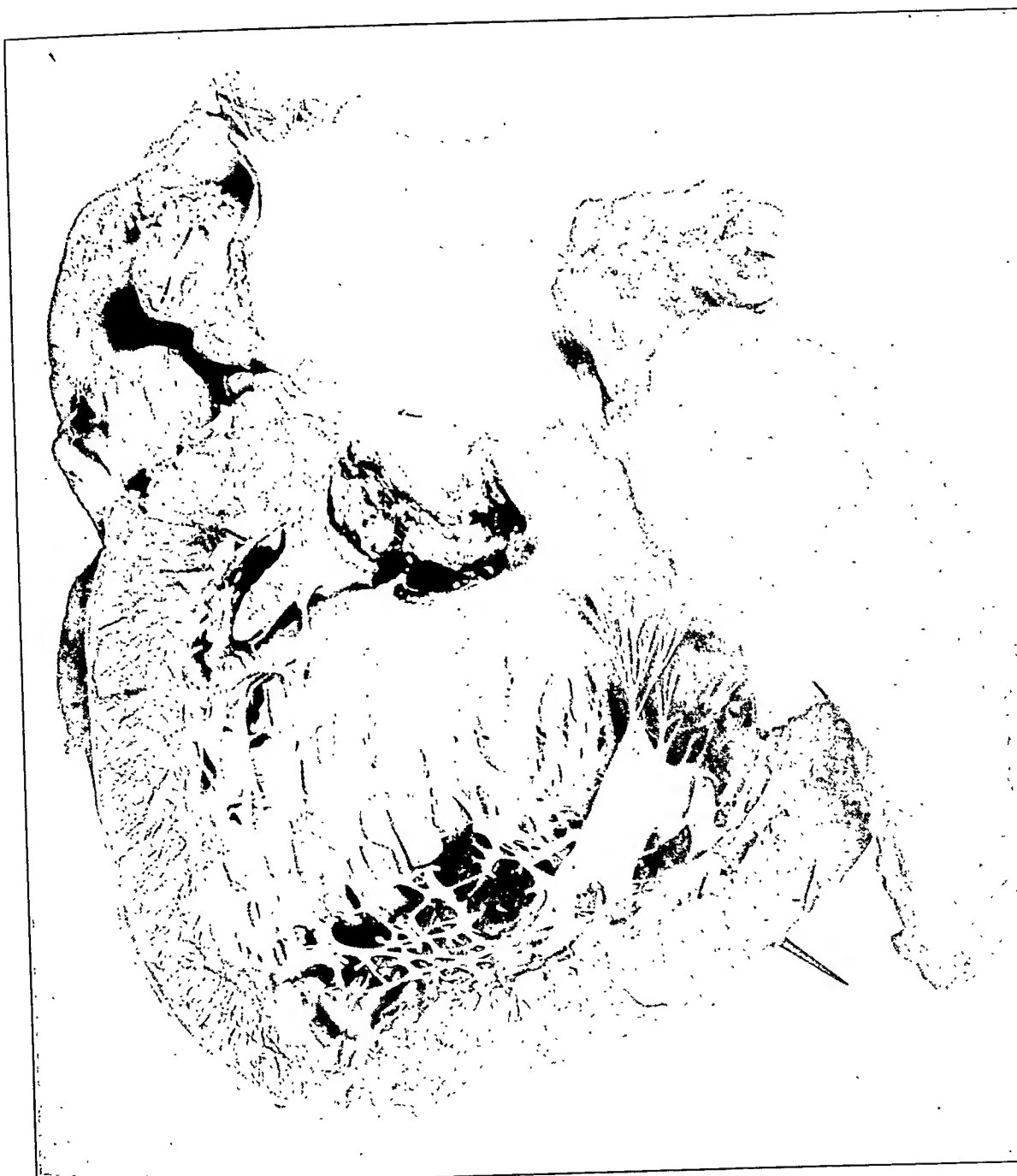
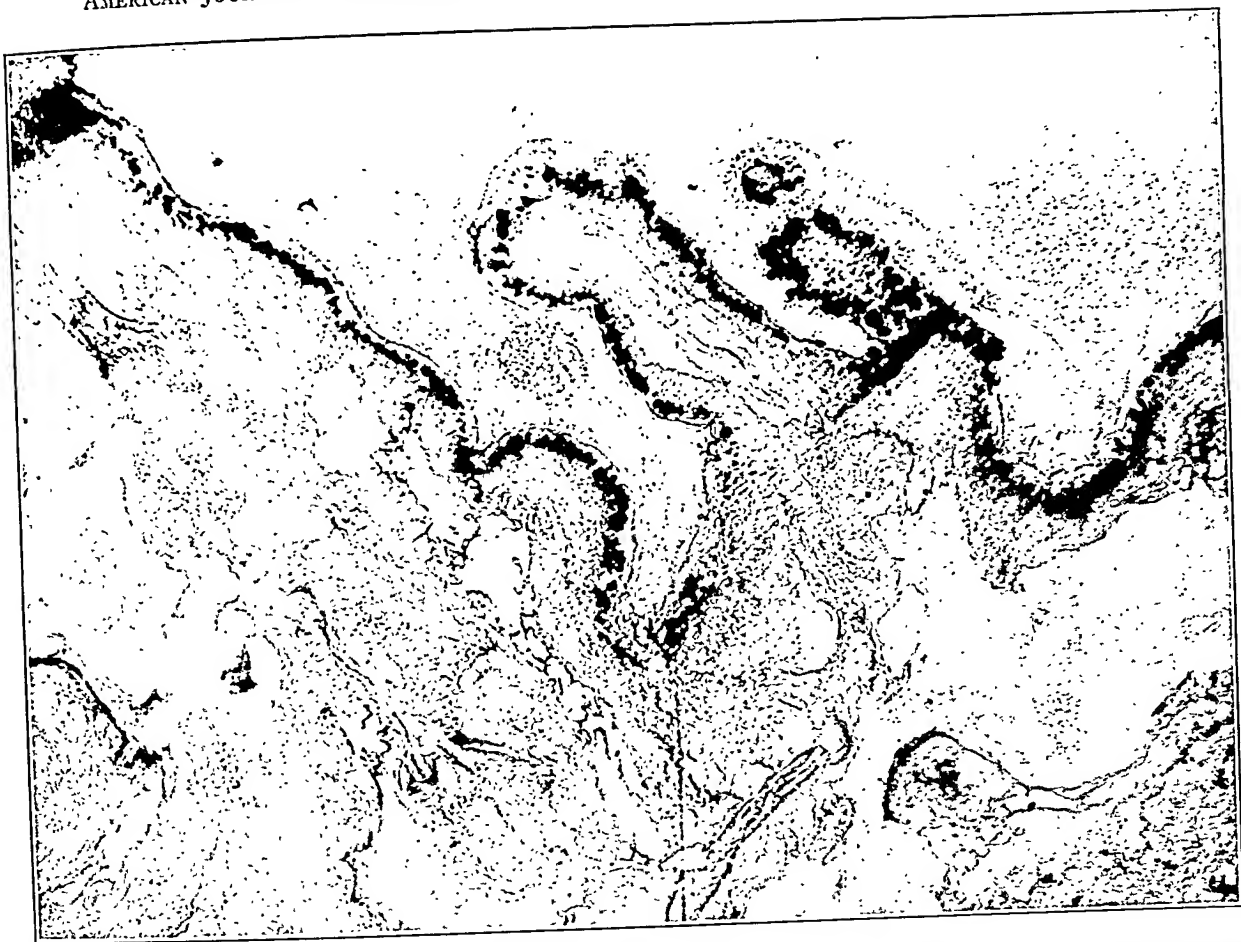


PLATE 164

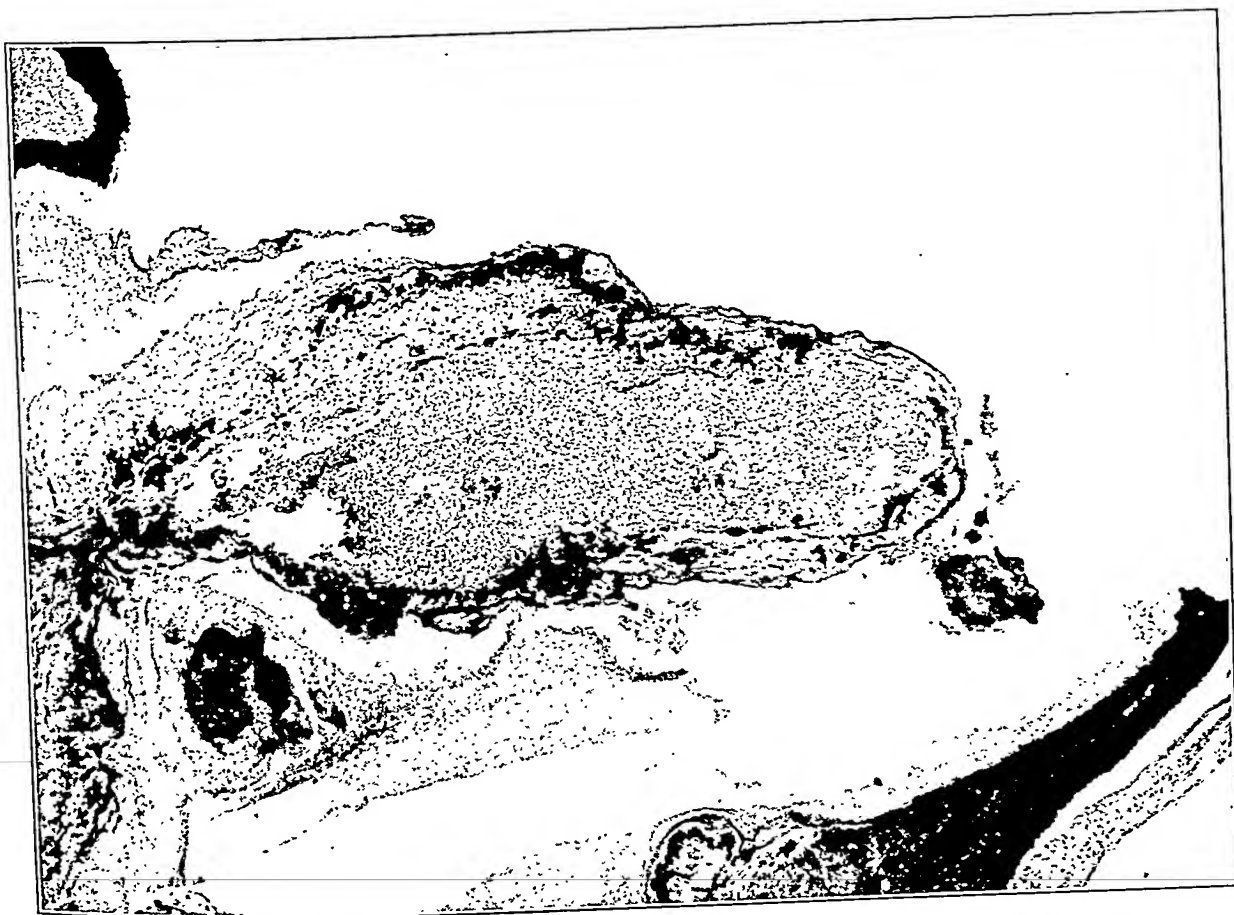
FIG. 5. Photomicrograph showing two polymorphonuclear leucocytes containing phagocytosed Gram-negative diplococci. From an area where ulceration has allowed the escape of leucocytes from a softened vegetation. $\times 1500$.

FIG. 6. A field in a suppurating area showing many phagocytic organisms in polymorphonuclear leucocytes. $\times 1500$.

FIG. 7. Section through the base of a vegetation showing masses of organisms, many in phagocytic cells. $\times 1500$.



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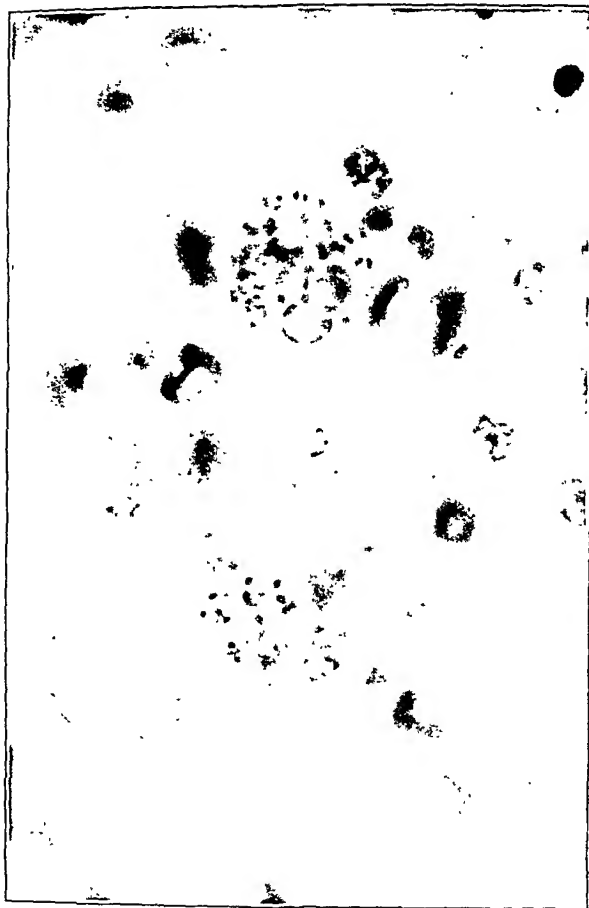
Rhoads

Meningococcus Endocarditis

PLATE 165

FIG. 8. Section through the heart muscle near the vegetations. It shows many necrotic muscle fibers and massive infiltration with polymorphonuclear leucocytes. $\times 250$.

FIG. 9. Section taken through the muscle of the left ventricle near the coronary artery. Endothelial leucocytes are shown grouped around a strand of necrotic collagen. This formation strongly suggests an Aschoff body. $\times 500$.



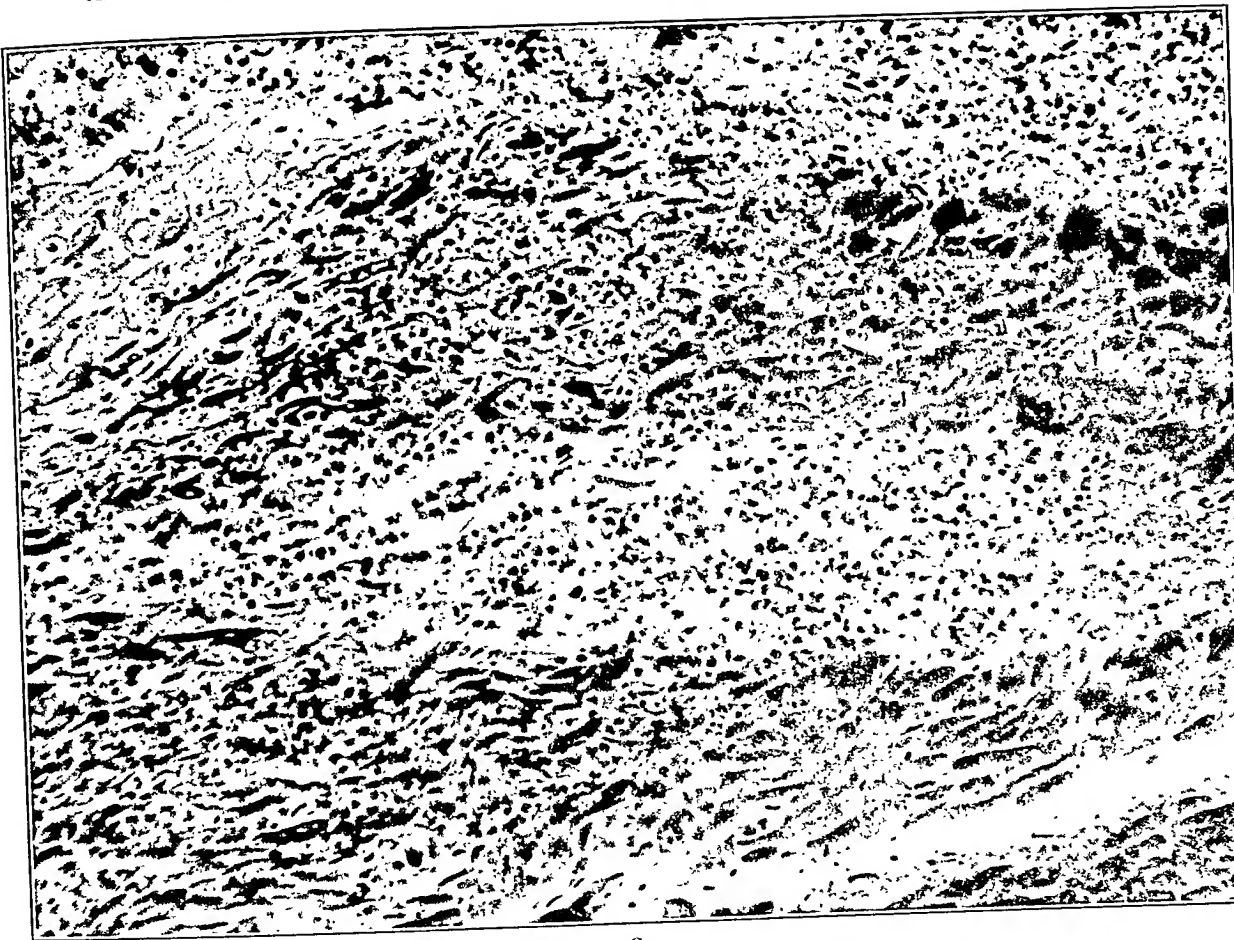
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8



9

Rhoads

Meningococcus Endocarditis

As a result of the replacement of handwork by machinery, this source of the disease became extinct (Teleky²).

According to whether the silver absorbed is deposited locally at the site of entrance, or carried through the entire body following entry into the blood stream and lymphatic system, argyria may be classified respectively as (a) local, or (b) generalized. The pathologic characteristics of local argyria are outside the scope of this paper. In generalized argyria the portal of entry of silver is usually the gastro-intestinal canal. Although, theoretically, silver may enter the blood stream from direct absorption through the mucous membranes, it is questionable whether the amount absorbed this way is ever sufficient to give rise to the clinical picture of generalized argyria. Argyria following the repeated local use of silver compounds in nose and throat conditions is the result of ingesting part of the silver. Menzel³ states that following the local application of silver preparations, argyria is always caused by the portion of silver absorbed through the gastro-intestinal canal. In support of this statement he points out that generalized argyria never occurs after long continued use of silver over the conjunctiva, or following local disseminated argyria, observed in silver workers. This is explainable by the fact that in these instances sufficient silver does not enter the body because of the small size of the absorbing surface. That generalized argyria may develop as a result of direct surface absorption is shown by Olshausen's case⁴ in which bluish discoloration of the buccal mucous membrane developed two months following the treatment of extensive burns with 0.1 per cent silver nitrate. In an individual reported by Crusius⁵ generalized argyria developed five months after the beginning of the treatment of a burn with silver nitrate. These are the only two instances reported in which generalized argyria developed as a result of absorption of silver through the body surface. The development of argyria following intravenous administration of silver compounds is not proved. In an instance noted by Tobler⁶ collargol was given by mouth and by intravenous injection. In a case discussed by Schlossberger⁷ no evidence is presented that the suspected argyria resulted from the intravenous administration of silver-salvarsan.

Generalized argyria may develop also after the use of organic silver preparations, provided sufficient silver is absorbed. There are five such instances reported in the literature, Crispin,⁸ Tobler,⁶

A CONTRIBUTION TO THE PATHOLOGY OF GENERALIZED ARGYRIA WITH A DISCUSSION OF THE FATE OF SILVER IN THE HUMAN BODY *

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The source of silver, found in rare instances in the human body, is therapeutic or occupational. Since the middle of the seventeenth century, when Angelus Sola advocated the therapeutic use of silver in epilepsy, tabes and chorea minor, silver nitrate has been frequently administered internally. Although keener physicians of the nineteenth century recognized the inefficiency of this treatment silver nitrate continued to be used in large doses in the conditions stated and, in addition, it was advocated in ulcerative lesions of the stomach. Lately the administration of silver nitrate internally has been abandoned. Local applications, however, still have considerable therapeutic value. In recent years various organic silver preparations have gained widespread use, especially in the treatment of certain nose and throat conditions. During such use, part of the drug is swallowed and, subsequently, through prolonged administration, a considerable amount of silver may gain entrance to the body by gastro-intestinal tract absorption. Aside from medicinal use, silver may enter the body by exposure in industrial processes when small particles accidentally penetrate the exposed skin surface of silver workers ("local disseminated argyria"). The dust of silver workshops may be the source of considerable amounts of silver in the body (Koelsch¹). It has been assumed that the silver dust-becomes absorbed both through the respiratory and the gastro-intestinal systems. Formerly one of the most common sources of industrial generalized argyria was the use of silver nitrate solution in the preparation of silver glass beads. In this process the silvering was performed by mouth suction; and by accidental swallowing of part of the solution generalized argyria resulted, which was, therefore, a common disease in Bohemia where this industry flourished.

* Received for publication August 1, 1927.

occurred, and he suggested that the deposited silver may act as an irritating foreign body. It is questionable whether this suspected relation plays any rôle in generalized argyria. The reported fatal accident following the intravenous administration of collargol (Tobler⁶) cannot be attributed to silver itself (Koller-Aeby¹³). Various diseases observed in patients suffering from generalized argyria are probably accidental and it is doubtful whether silver is a predisposing factor. The first appearance of generalized argyria occurs usually over the edges of the gingiva in the form of a bluish violet margin. The location of this line is similar to that accompanying lead poisoning. There is considerable practical importance connected with this early manifestation of argyria, as the development of a "silver line" is an absolute contraindication for the continuation of the therapeutic use of silver. Following the appearance of the blue color over the gums, similar discoloration develops over the mucous membranes of the mouth, conjunctivae, sclerae and skin of the face. Gradually, then, depending on the amount of silver absorbed, the entire skin surface of the body changes color, though the exposed areas are more intensely blue than the covered surface of the skin.

The diagnosis of generalized argyria does not offer difficulties if a positive history is available, and if the change in the color of the skin is definite. Particularly if a history of taking silver is not available, early cases offer considerable difficulty in differentiating the disease from hemochromatosis and from methemoglobinemia (Firth and Harrison,¹⁴ and Harrison¹⁵).

Charcot's statement that silver is eliminated by the kidneys was not confirmed by later observers. There is a possibility that traces of silver may be eliminated through the intestines.¹⁶ All the available data agree, however, that most of the silver absorbed becomes permanently deposited in the tissues. Some caution is necessary in the interpretation of observations in man, as in the majority of instances observations as to the elimination of silver were performed after the patients ceased taking silver. It is for this reason that the question of elimination of silver during the period of intake is an open problem. Animal experiments do not throw light on the fate of silver in man, since it has not been possible to produce in animals a condition corresponding to human argyria.

Because silver once deposited becomes permanently fixed in the

Goldstein⁹ and Davis,¹⁰ and we know of an additional unreported case in Boston. In all of these cases, silver preparations were used locally in the nasopharynx, and undoubtedly large amounts of silver entered the blood stream through gastro-intestinal absorption. As the antiseptic and astringent properties of organic silver compounds do not excel those of other chemicals, the prolonged use of organic silver preparations in the nasopharynx should be avoided.

The minimal amount of silver necessary for the production of the clinical picture of generalized argyria probably varies considerably. The determination of this amount as obtained from statements by patients gives only an approximate idea. Koelsch¹ quotes a case in which two grams of silver nitrate administered within two months produced argyria. This amount is unusually small. Krysztalowitz¹¹ estimated that at least twenty-five to thirty grams of silver nitrate have to be taken within a half year before the clinical picture of argyria develops. Formerly, epileptics and tabetics often received seventeen to thirty-four grams of silver nitrate without any obvious effects. A critical review of the cases reported in the literature indicates that, just as in poisoning with other heavy metals (manganese, lead, mercury), considerable variation exists between the dosage and the toxic effects produced. The predisposing factors for susceptibility and resistance are largely unknown at the present time.

The frequency of generalized argyria cannot be determined with any degree of accuracy. The cases reported in the literature do not give a correct index of the frequency of the disease. Generalized argyria was once a rather common disease. With the change in the therapeutic application of silver nitrate, and with the progress of preventive industrial medicine, however, generalized argyria became rather a disease of the past. With the recent widespread use of organic silver preparations a few new instances of the disease have been observed.

Except for the appearance of the skin, silver *per se* probably does not produce clinical symptoms and signs. Although Menzel³ stated that some of the patients develop fatigue, restlessness and loss of appetite as a result of the withdrawal of silver following its long use, it is probable, as Koelsch¹ pointed out, that these symptoms are psychic in origin. Blum¹² observed that following the local use of argyrol in the bladder, malignant growths of the mucous membrane

Kino²⁷ and others attributed a specific reducing capacity for silver to connective and especially elastic tissue. As a result of reduction the silver compound becomes precipitated.

We do not possess any therapeutic agent which may mobilize or influence the clinical picture of generalized argyria.

Because of the scarcity of careful histologic studies on generalized argyria and because the histologic and chemical observations on the case to be reported indicate that the patient was suffering from the most marked degree of argyria reported in the literature, we believe that the report of our study is of value.

REPORT OF CASE

The patient was about seventy years of age, and one of the curiosities of the Barnum and Bailey circus. For many years he was presented to the public as the famous "blue man." The patient's own story both in the circus and in the hospital was that he was suffering from congenital heart disease which suddenly became worse when he fell from a horse, while fighting as an officer in the British army in India. Although repeated attempts were made to obtain a history to show therapeutic use, or industrial contact with silver, no positive statement could be elicited. On Aug. 13, 1923 he was admitted to the Second Medical Division of the Bellevue Hospital. There was considerable difficulty in obtaining a history as he answered questions reluctantly, was suspicious and resented attention. He complained of dyspnea and orthopnea of four weeks duration and had suffered from cough for the past year. For three weeks the cough had been more intense. For some time he had suffered from nocturia three times a night. For years, walking had been difficult and unstable. His wife, who had known the patient for thirteen years, stated that during that period the color of his skin had not changed and he had only occasionally taken medication of any kind. A friend, who had known the patient for thirty years, had observed the same color since the first meeting. He gave the information that the patient had worked in silver mines. He had seen a photograph of the patient taken about thirty-five years before which showed his skin to be white.

On physical examination he was a thin old man of seventy to seventy-five years, who appeared dyspneic and orthopneic. He was in acute distress and the examination was difficult. The hair over

tissues and because animal experimentation does not simulate human argyria, knowledge regarding the behavior of silver in man has been obtained from studies of postmortem material.

It has been possible to gather from the literature twelve cases of argyria with postmortem examinations (Fromman,¹⁷ Riemer,¹⁸ Liouville,¹⁹ Neumann,²⁰ Weichselbaum,²¹ Dittrich,²² Jahn,²³ v. Fragstein,²⁴ Moslener,²⁵ Dohi,²⁶ Kino,²⁷ and Tobler⁶). The majority of these studies are somewhat incomplete and practically all our knowledge of pathology is based on Fromman's, Riemer's and Kino's contributions. Their investigations showed that silver becomes deposited in small, and nearly even-sized granules in the tissues. The microchemical behavior of the granules indicates that they consist of an organic silver compound, probably silver albuminate. It is stated that with the exception of brain and cord silver becomes deposited in every organ of the body. It is characteristic that silver has a special affinity for connective tissue and particularly for elastic fibers. The specific cells of organs do not contain silver, but the latter is deposited in the supporting connective tissue. The pigment deposit is most marked in the lymph nodes, in the glomeruli of the kidneys and in the choroid plexus of the ventricles. The epidermis of the skin is always free from silver. Just below the epidermis, however, and along the elastic fibers adjacent to the sweat glands, sebaceous glands and hair follicles, rich deposits of silver are usually present. In contrast to other foreign bodies, it is characteristic that silver deposits do not call out cellular reaction. According to Kino,²⁷ the silver granules are always extra-cellular.

In only two instances was there an attempt to determine quantitatively the amount of silver in some of the organs. Fromman¹⁷ found 0.047 per cent of metallic silver in the dried tissue of the liver and 0.061 per cent in the dried tissue of the kidneys. Jahn²³ found 0.018 per cent silver in the fresh liver and 0.03 per cent silver in fresh kidneys.

The exact mechanism by which silver deposits are formed is not known. Fromman¹⁷ describing his histologic studies on the first postmortem examination of argyria reported in the literature suggested that silver nitrate during its intestinal absorption changes to silver albuminate, which becomes deposited in the walls of the vessels following its loss in solubility. Riemer¹⁸ thought that the connective tissue acts as a mechanical filter in retaining silver.

costal margin. The patient's general condition became gradually weaker and he died seven days after admission.

A postmortem examination of the body was performed Aug. 20, 1923 by Dr. Douglas Symmers with the following findings:

NECROPSY REPORT

The body is that of a man 68 years of age, 167 cm. in length, of moderately large, well developed frame, poor musculature and nutrition. The skin of the entire body is an intense slate-blue color, with a peculiar lack of luster. The color is not modified by pressure. The skin of the head, face and neck is more intensely pigmented than that of other parts of the body. The conjunctivae present the same intense bluish gray pigmentation as the mucous membrane of the mouth. The pupils are semidilated and are even in size and outline. There are no palpable, enlarged superficial lymph nodes. The muscles of the thenar and hypothenar eminences on both sides are distinctly flattened. The interosseous spaces on the backs of both hands are noticeably deepened. The muscles of both arms and forearms and of both thighs are flabby and atrophied. The pretibial tissues are edematous, as are those of the dorsum of the foot, and pit easily on pressure. The left testicle, palpated through the scrotum, appears to be of firmer consistence than that of the opposite side.

On section, the subcutaneous fat is almost completely absent. The muscle tissues of the thoracic and abdominal walls present a dull, brownish red color. The fascial tissues, however, in these vicinities present a distinct silvery sheen. The muscles are atrophic, the individual muscle bundles separating easily.

Abdominal Cavity: The peritoneum is smooth throughout, but presents a distinct diffuse light bluish discoloration. The serosa of the intestines, both large and small, presents a diffuse slate-gray pigmentation. The diaphragm is placed normally.

Chest: On opening the thoracic cavity, no thymic remains are visible. Both pleural cavities are dry and free from adhesions. The anterior edges of the lungs meet in the midline, obscuring the upper half of the precordial area. The precordial fat is atrophic.

Heart: On opening the pericardium, there is no excess of fluid and the membrane is smooth and glistening throughout. The peri-

the scalp and the mustache were glistening white. The color of the skin was of an unusually deep blue and from a distance appeared almost black. This deep color was almost uniform throughout the entire body, although it was more intense over the exposed skin areas. The sclerae were also of a dark blue color. The pupils were contracted, small, equal and regular. They reacted sluggishly to light and the accommodation test could not be performed. Examination of the eye-grounds showed tortuous dark vessels with extensive dark bluish areas covering the eye-grounds almost completely. The patient was totally deaf on the right side and partially on the left. The mucous membranes of the mouth were similarly dark blue. The tongue was coated and dry.

The neck was normal. The respirations were labored, shallow and rapid. Anteriorly the resonance, fremitus and breath sounds were normal. Posteriorly there was dulness over both bases with numerous fine crepitant rales. The heart was slightly enlarged (11.5 cm. in 5th space). The sounds were feeble, rapid and totally irregular. The systolic murmur heard over the apex was not transmitted to the axilla. The brachial, radial, temporal and femoral arteries were tortuous and thickened. The pulses were equal, totally irregular, and there was a pulse deficit of 20. The blood pressure was 110 systolic and 80 diastolic. The temperature fluctuated between 98° and 102.8° F. The abdomen was slightly protuberant. Tenderness was observed over the epigastrium. There was pitting edema of the sacral region and over both ankles. The deep reflexes were unusually sluggish, the neurologic examination being otherwise normal. The muscles were poorly developed or perhaps atrophied. The thenar and hypothenar eminences were flattened.

The hemoglobin content of the blood was 80 per cent. The red cells were normal in appearance with no stippling. The specific gravity of the urine was 1028 with acid reaction. There was a trace of albumin with an occasional granular cast in the urine. No red cells were present. The urine did not reduce Benedict's solution.

The diagnoses of generalized argyria, generalized arteriosclerosis, myocardial degeneration, auricular fibrillation and severe circulatory failure with bronchopneumonia were made. The possibility of primary muscular atrophy was suggested.

During the following week the patient developed Cheyne-Stokes respiration. The liver edge became palpable three fingers below the

Kidneys: The left kidney is surrounded by a moderate amount of fat. On removal, the organ appears to be slightly diminished in size. Seen through the capsule, it presents a bluish gray tinge. On section, it cuts without noteworthy resistance. The capsule strips readily and leaves behind a smooth surface in which there is a suggestion of a grayish tinge. On section, the cortex and medulla are well defined and well proportioned. The cortical markings are indistinct. Viewed by reflected light, the glomeruli stand out as sand-like bodies. In the upper pole of the kidney, on the left side, and immediately under the capsule, is a whitish, rather firm, rounded, well circumscribed mass, which measures about 1.5 cm. in diameter, one-half of which projects above the surface of the kidney; the other half replaces the substance of the organ in the immediate vicinity.

The right kidney is essentially the same as the left with the exception that it contains no nodular mass.

Liver: The liver is diminished in size. The capsule presents a diffuse bluish gray discoloration. On section, the organ cuts readily; the lobules stand out fairly distinctly as brownish red islands. The cut ends of the vessels throughout the substance of the liver are distinctly bluish gray in color. The intima of the larger veins, when laid open, is perfectly smooth but strikingly silvery in color. The gall bladder contains a quantity of dark blackish bile and some thirty or forty small, greenish brown, faceted stones. The mucous membrane of the bladder is atrophic, otherwise apparently well preserved. The larger bile ducts show no noteworthy changes.

Stomach: The serous covering of the stomach is diffusely silvery gray in color. On opening the stomach, the mucous membrane appears well preserved, except that the rugae in places have disappeared and in other places are atrophic. No focal lesions are visible.

Intestines: The serous coat of the intestines is distinctly silvery gray, but the mucous membrane appears to be well preserved.

Retroperitoneal Lymph Nodes: These are scanty in number, small in size and are apparently free from pigment.

Aorta: The abdominal and thoracic aortae present no changes other than those already mentioned.

Bladder: The urinary bladder is small and contracted. On section, the walls appear slightly thickened. The mucous membrane is thrown into a number of fine corrugations and is silver-colored throughout its entire extent.

cardial tissues present a faint slate-bluish appearance. The heart is distinctly increased in size; the right side is markedly distended. The apex is rounded. On opening the right side of the heart, an immense quantity of thin, deep bluish red fluid blood escapes. The auriculoventricular opening on this side easily permits the entrance of three fingers. On opening the left side of the heart, a large quantity of red fluid blood escapes from the auricle. On this side the auriculoventricular opening is likewise easily permeable to three fingers. All the septa are intact. The endocardium on both sides of the heart presents a diffuse silvery blue appearance. The muscle tissue is a dirty dull brownish red, in which there is a silvery tint. The endocardium covering the papillary muscles in the left ventricle, and that of the posterior wall of the ventricle just beneath the aortic opening, is thickened and of a rather deeper bluish gray color than that of the rest of the ventricular endocardium. The valves throughout the heart show no indication of thickening. In both the aortic and mitral valves, however, are patches of silver colored pigment. The subendothelial tissues of the pulmonary artery and aorta present large, irregularly outlined, ill-defined areas which are of a silvery hue. The caliber, thickness and elasticity of the aorta and pulmonary arteries appear to be entirely normal. The heart muscle at the attachment of the posterior mitral leaflet is about 1.5 cm. in thickness. The heart as a whole is flabby and weighs 480 gm.

Lungs: The left lung is removed without difficulty. The pleura is smooth and glistening throughout. There is no excess of fluid in the pleural cavity. The left lung is fairly voluminous. Seen through the pleura, it is diffusely reddish blue in color. The pleura appears to be entirely free from pigment. On section, the organ cuts readily. The upper lobe is feathery to the touch and dry. The substance is apparently well preserved. The lower lobe, on pressure, exudes a moderate amount of frothy serum. The peribronchial lymph nodes are small and blackish in color. The right lung is essentially the same as the left.

Spleen: The spleen is normal in size and moderately diminished in consistence. On section the cut surface is smooth, bluish red, the substance abundant and the markings indistinct. No lymphoid follicles are visible. In the cut ends of the blood vessels one gains the impression that there is a certain amount of silvery discoloration.

Suprarenal Capsules: These appear unchanged.

THE METHOD FOR ISOLATION AND IDENTIFICATION OF THE SILVER

The tissue was ground, strongly acidified with nitric acid, dried, and then ashed at dull red heat. In this connection it may be pointed out that the ash may contain the silver as water soluble salts (silver nitrate, silver sulphate) and ammonia soluble salts (silver chloride, silver phosphate) and nitric acid soluble metallic silver. The ash was leached, successively, with hot water, ammonia, and 50 per cent hot nitric acid in order to get all the silver into solution. The three filtrates were combined and hydrochloric acid was added to them until all the silver was completely precipitated as AgCl . The precipitate was filtered and washed with water acidulated with nitric acid, until the filtrate was free of chlorides.

Confirmatory Tests

1. A white curdy precipitate, soluble in ammonia, in potassium cyanide and sodium thiosulphate was obtained by the above precipitation.

2. The white precipitate changed to bluish violet on exposure to light.

3. Some of the white precipitate, dissolved in ammonia, was treated with H_2S ; a black precipitate resulted which was insoluble in ammonia or in HCl .

4. Some of the ammoniacal solution of the white precipitate was precipitated by H_2S ; the resulting black precipitate was dissolved in hot nitric acid and evaporated to dryness. This was taken up in water and separately tested with

- (a) Potassium iodide — yellow precipitate
- (b) Potassium chromate — blood-red precipitate
- (c) Sodium phosphate — yellow precipitate
- (d) Potassium ferrocyanide — white precipitate
- (e) Potassium ferricyanide — brownish red precipitate

The foregoing tests conclusively prove the presence of silver in the tissues.

THE QUANTITATIVE DETERMINATION OF THE SILVER

Weighed amounts of the various organs listed in the Table below were acidified with nitric acid, then dried, and ashed in an electric

Prostate: Except for the same silvery discoloration, the prostate appears to be well preserved.

Testicles: The right testicle on removal is found to be somewhat diminished in size. The tunica vaginalis is smooth, glistening and bluish in color. On section it cuts readily and the tubules string out easily. The cut surface is a deep mahogany color. The left testicle is decidedly smaller than normal. Its consistence is noticeably increased. On section, it cuts firmly and the cut surface presents numbers of streak-like areas which are firm to the touch and distinctly silvery in color. Approximately two-thirds of the testicular substance is thus irregularly replaced by the overgrowth of fibrous tissue.

Pancreas: The organ is long and narrow and on section presents a faintly yellowish color in which there is a distinct suggestion of grayish blue. The cut ends of the vessels, wherever seen, are distinctly bluish gray in color.

Brain: On removing the calvarium, the dura mater presents a silvery appearance. On lifting the dura, the convolutions, particularly in the frontal and parietal lobes, appear markedly diminished in size and the sulci are deepened. The vessels at the base are well preserved. On section, the substance of the brain shows no focal lesions, but both the white and gray matter have a suggestion of silvery pigmentation. The choroid plexuses are deeply pigmented, presenting a dark silvery color, almost black. The venous and osseous sinuses show no noteworthy changes, although the lining membranes of the former are silvery in color.

Anatomic Diagnoses: Generalized argyria with pronounced silvery pigmentation of the heart, including the endocardium and the pericardium; peritoneum, kidneys, liver and pancreas, particularly their blood vessels; and of the pulmonary artery and aorta; dura mater and brain, including excessive deposits in the choroid plexuses. Chronic interstitial obliterative orchitis (left side).

CHEMICAL EXAMINATION

The results of the chemical examination warrant attention because of the extremely high silver content of the organs. The quantity of silver found in this instance is far in excess of that found in the two cases mentioned above (Fromman,¹⁷ Jahn²³), in which the kidneys and liver only were tested.

HISTOLOGIC EXAMINATION

Methods: The tissue was fixed in Zenker's fluid and embedded in paraffin. The sections were stained in hematoxylin and eosin, eosin-methylene blue, phosphotungstic acid hematoxylin, Verhoeff's and Weigert's elastic tissue stains and Stewart's method for silver in tissues.

Heart: In the epicardium silver is present in small granules of uniform size and is scattered diffusely through the connective tissue. Next to the muscle where the connective tissue is loose the granules are most plentiful and they form an irregular line. They tend to be arranged in rows along the bundles of collagen fibers and follow rather closely the distribution of the elastic fibers.

The myocardium contains no silver in the muscle fibers. There are granules scattered along the collagen fibers of the intermuscular connective tissue and occasionally grouped in masses larger than a single cell. The silver tends to be most prominent where elastic fibers are most plentiful.

The arteries show a row of silver granules along the inner elastic membrane. In the muscular coat there are a few granules scattered through the connective tissue between the muscle fibers. In the adventitia there is a dense row of silver granules just outside the outer elastic membrane. The veins show rare granules except in the adventitia where a narrow row is formed. In the capillaries a thin line of granules is present in the connective tissue just under the endothelium. The vessels of the heart contain less silver than those of any other organ except the brain.

In the nerves the metal lies in scattered granules between the collagen fibers of the nerve sheath.

Lung: In the pleura, through the loose connective tissue just under the mesothelial surface, the granules of metal are distributed irregularly. As the connective tissue becomes denser near the lung parenchyma, the granules group to form a line along a heavy strand of elastic fibers, some of which appear to be beaded with silver granules contained in the fiber itself.

The alveoli show silver scattered through the connective tissue situated among collagen fibers, often close to elastic fibers. There is none of the metal in epithelium.

The arteries and veins show very few granules distributed through

furnace at a dull red heat. The ash was then leached with hot water in order to extract all soluble silver salts. It was next leached with ammonia water in order to dissolve any silver chloride and silver phosphate. Finally, it was digested with hot nitric acid to dissolve all metallic silver. The three solutions thus obtained were combined into one. The combined extracts, if not alkaline, were made so by adding ammonia. Hydrochloric acid was then added gradually, with stirring, until all of the silver was precipitated. The final solution was made slightly acid with HCl. It was then placed in a boiling water-bath for one hour and left standing in the dark for twenty-four hours. The precipitate was then filtered and washed several times with water containing a little nitric acid, then redissolved by ammonia and the solution treated with H_2S . The resulting precipitate of silver sulphide was filtered off and washed with water. It was then redissolved in concentrated nitric acid and the solution evaporated to dryness on a boiling water-bath. The residue was next dissolved in water and, if not perfectly clear, it was filtered; the filter paper was washed thoroughly and the washings added to the main solution. This solution was now acidified with nitric acid and brought to a boil. Hydrochloric acid was then added drop by drop, with stirring, until all the silver was completely precipitated. After standing in the dark for twenty-four hours the precipitate was filtered through a Gooch filter, washed with water containing a little nitric acid, until the filtrate showed the absence of any HCl, and finally washed twice with alcohol. The washed precipitate in the Gooch filter was then dried at $100^{\circ} C$, and finally at $130^{\circ} C$, until a constant weight was obtained.

The tabulated results of the quantitative analyses of the tissues follows:

Silver Content of Tissues

	Amount analyzed grams	Silver (Ag) recovered milligrams	Per cent Ag content per cent	Total Ag content grams
Kidneys.....	40	97.5	0.24	0.73
Heart.....	122	181.5	0.15	0.46
Brain.....	149	15.7	0.011	0.15
Liver.....	104	73.0	0.07	1.35
Muscles.....	35	54.2	0.16	45.7
Bone.....	62	130.0	0.21	24.4
Gall Stones.....	0.5	Negative	Negative	Negative

are marked out by narrow rows of granules. The vessels commonly show the arrangement of a few granules along the inner elastic membrane and a greater number in the adventitia near the outer elastic membrane.

Liver: In the capsule, the silver granules are scattered through the connective tissue in close association with elastic fibers. In the part of the capsule which lies next to the liver the particles are more numerous and form a rough line without apparent relation to the number of elastic fibers present.

The connective tissue about the portal areas shows a large number of silver granules scattered in close proximity to the elastic fibers. Here and there are a number of endothelial leucocytes which have engulfed one or more granules of the metal.

There is no silver in the liver cells. There are granules scattered in the strands of fibrous tissue which form the supporting structure. No metal is seen in Kupfer cells.

The arteries have a few particles in the connective tissue under the endothelium and a larger number along the external elastic membrane. In the veins nearly all the granules are in the adventitia. The ducts have a large amount of silver scattered in the surrounding connective tissue. Here there is a definite relation to the elastic fibers.

Kidney: Through the connective tissue of the capsule there are scattered granules of silver which have some tendency to follow the distribution of the elastic fibers. The tubular epithelium contains no silver. The fibrous tissue between and around the tubules contains a large number of granules. The large vessels contain very little of the metal. The walls of the glomerular capillaries contain a very marked deposit of silver. The metal is arranged in rows of granules in the delicate layer of connective tissue under the endothelium. In some places it is difficult to say that the metal is not actually in the endothelial cells. The capsular epithelium is underlaid by a narrow row of silver granules following the collagen fibers of the connective tissue.

Bladder: The epithelium contains no silver. In the submucosa there are isolated granules of metal deposited throughout. About midway between the epithelium and the muscularis the particles are massed in an irregular line. There is no relation between this line and the distribution of the elastic fibers. Through the con-

the adventitia. In the alveolar capillaries the silver forms a narrow line just under the endothelium.

The bronchioles show only an occasional granule of silver in the connective tissue.

Spleen: The connective tissue of the capsule and trabeculae contains a moderate amount of silver scattered in fine particles along the bundles of collagen fibers. Along the fine fibers of the supporting reticulum of the organ the silver granules are arranged in rows, in some places marking out the fibers clearly. There are a few free phagocytic cells which contain masses of silver. Elsewhere the metal is entirely extracellular.

The vessels contain a large amount of silver. It is arranged with a few granules scattered along the internal elastic membrane and many particles quite densely packed among the elastic fibers near the adventitia. In the vessels of the spleen the metal deposit is more marked along the outer elastic membrane than in the vessels of other organs.

Pancreas: The connective tissue around the pancreas and forming the supporting structure contains isolated granules of silver irregularly distributed along the bundles of collagen with some relation to the elastic fibers.

The cells of the acini and of the islands do not contain silver. The ducts show the metal granules scattered through the fibrous tissue. The granules lie in close relation to the elastic fibers.

In the arteries the silver forms a thin line under the endothelium in connective tissue. There are a few granules between the muscle fibers and a heavy line of metal particles next to the outer elastic membrane. The veins show a fair amount of the material scattered through the adventitia.

Intestine: The epithelial cells contain no silver. Distributed through the connective tissue, between the glands, are a few granules. In the region just below the epithelium where the fibrous tissue becomes densest the particles of silver are arranged in an irregular line. The elastic fibers are not particularly numerous in this region.

Throughout the submucosa the silver granules are scattered diffusely tending to form narrow rows along the bundles of collagen, and are often in or beside elastic fibers. In the muscularis the particles follow the connective tissue as it ramifies about the muscle fibers. The capsules of sympathetic ganglia and sheaths of nerves

DISCUSSION

The pathologic, histologic and chemical findings reported in this communication indicate that the patient suffered from the most severe degree of argyria ever reported in the literature. Unfortunately, the history did not indicate the amount and the form of silver taken by the patient. The clinical signs and symptoms exhibited by the patient cannot be related to argyria with certainty. The unstable gait and atrophy of the small muscles of the extremities may have some relation to the silver. Until similar additional observations are available, however, such a relation cannot be established. We wish to call special attention to the results of the chemical analysis of the tissues on account of the extremely high silver content. If we accept that the muscles represent 43.4, the bones with the ligaments 17.5, the liver 2.75, the brain 2.16, the kidneys 0.46, and the heart 0.46 per cent of the body weight (Vierordt²⁸) then on the basis that the body weight of this patient was 66,400 gm. we estimate the silver content of the musculature was 45.7 gm., that of the skeleton 24.4 gm., of the liver 1.35 gm., of the kidneys 0.73 gm., of the heart 0.46 gm., and of the brain 0.15 gm. From these results a conservative estimate indicates that the silver content of the body was about 90 to 100 gm.

The histologic studies reveal that the detectable silver is present in two types of tissues: (a) in connective tissue, and (b) within phagocytic cells. In the connective tissue the silver rarely if ever is within the cell. The granules group themselves in rows along the bundles of the collagen and occasionally are in close proximity to the elastic fibers. Whether they are only along the surface, or actually within the cell cytoplasm, cannot be stated with certainty. In the fibrous tissue underlying the epithelial-lined surfaces, the silver particles are arranged where the collagen bundles are loose, without relation to elastic fibers. There is a definite tendency for the silver, as observed by other investigators, to form rows of granules in the connective tissue underlying secreting epithelium and the endothelium of vessels. In these regions there may be some relation to the presence of elastic fibers. The wandering phagocytic cells contain silver particles within the cell, an observation indicating that silver particles in the human body are not always extracellular, as stated by other observers.

nective tissue among the muscle fibers there are silver granules along the collagen. The vessels and nerves show rare silver particles in fibrous tissue. There are a few phagocytic cells which contain granules of silver.

Aorta: A definite line of silver granules lies in the connective tissue between the intima and the media. Though there are a number of wandering cells present in the region, none is seen that contains silver. The muscular layer contains a few scattered silver granules in the connective tissue. In the adventitia the particles form an irregular line close to the media just external to the layer of elastic fibers. In the vessels of the adventitia only a few granules are present.

Brain: The choroid plexus shows a line of silver granules just under the epithelium. The particles are densely grouped in this region and it is difficult to ascertain whether or not they are in the epithelial cells. There is a small amount of silver scattered through the connective tissue. Underlying the capillary endothelium is a well defined line of granules. No silver is seen in the parenchyma of the brain. The large vessels show only a few granules in the adventitia. The capillaries are marked out by a narrow, dense line of silver particles just under the endothelium.

Skin: No silver is seen in the epithelium. In the corium there are a few irregularly scattered granules. The metal is also present in masses larger than a single cell, lying apparently quite free. Along the collagen fibers of the connective tissue adjoining the epithelium of the sweat and sebaceous glands are well defined lines of silver granules. In this region the metal is in close relation to elastic fibers.

Lymph Node: The capsule contains some scattered silver granules. Through the pulp, silver is distributed in rows of particles ranged along the supporting reticulum, in masses of clumped particles lying free in the sinuses, and is contained in phagocytic wandering cells.

To prove that the granular material present in the lymph node is silver and not carbon, concentrated nitric and hydrochloric acid was applied to the sections. This changed the color of the silver masses from dark brown or black to a light yellow, as seen under the oil immersion lens. Sections of a lung containing carbon were similarly treated without effect on the color of the pigment.

7. Histologic studies of the tissues indicate that the silver was present in (a) connective tissue, and (b) in phagocytic cells.

8. In the connective tissue the silver is rarely if ever within the cells. The granules have a tendency to become deposited along and between the bundles of the collagen and the elastic fibers.

9. The presence of silver granules within phagocytic cells of the spleen and lymph nodes is the only instance where the silver was observed within the cytoplasm of cells.

10. The deposited silver granules do not cause "foreign body" cellular reaction on the part of the body.

11. The affinity of silver for connective tissue and the unusually high silver content of bones suggest certain similarity between the behavior of silver, calcium and lead in the body. Among the heavy metals which may become deposited in the human body in relatively large amounts silver is of slight and perhaps least toxicity.

We are indebted to Dr. Douglas Symmers for the necropsy material obtained, to Dr. F. B. Mallory for the photomicrographs, and to Miss Lillian M. Leavitt for technical aid.

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The silver content of the brain was appreciably lower than that of other organs. Previous observers state that the brain and cord are the only organs not containing silver. While it is true that the silver is not deposited inside or outside of the nerve or glia cells in the brain, similarly, specific cells of other organs frequently do not contain silver. The deposit of silver in the vessels of the brain is in harmony with the general behavior of silver in the human body elsewhere. Whether the chemically detected silver corresponds to the amount of silver visible by histologic examination in the vessels, or whether beside the visible granular deposits, silver is present in other forms in the brain cannot be stated from the available data. It is of interest that on gross examination both the white and the gray matter had a suggestion of silvery pigmentation.

SUMMARY

1. Generalized argyria may follow the absorption of both organic and inorganic silver compounds.

2. Review of the literature indicates that generalized argyria following the repeated application of organic silver preparations (argyrol, collargol) is caused by the silver ingested and absorbed from the gastro-intestinal canal.

3. The earliest appearance of generalized argyria manifests itself on the gingiva, and over the mucous membrane of the buccal cavity. Bluish discoloration over the same area is absolute contraindication for the use of silver preparation.

4. Studies on a case of generalized argyria are presented. The pathologic, histologic and chemical observations on the case indicate that the patient was suffering from the most severe degree of generalized argyria ever reported in the literature.

5. Chemical analysis of organs revealed the following concentration of metallic silver in the organs: kidneys 0.24 per cent, bone 0.21 per cent, muscles 0.16 per cent, heart 0.15 per cent, liver 0.07 per cent and brain 0.01 per cent. The total silver content of the organs was: muscles 45.7 gm., skeleton 24.4 gm., liver 1.35 gm., kidneys 0.73 gm., heart 0.46 gm., and brain 0.15 gm. The total estimated silver content of the body was 90 to 100 gm.

6. Resembling the cases reported in the literature before, there is not sufficient evidence to indicate that the silver present in the body produced any signs or symptoms except discoloration.

DESCRIPTION OF PLATES

PLATE 166

FIG. 1. Photomicrograph of a glomerulus showing granules of silver among the collagen fibers of the connective tissue underlying the endothelium of the capillary. $\times 500$.

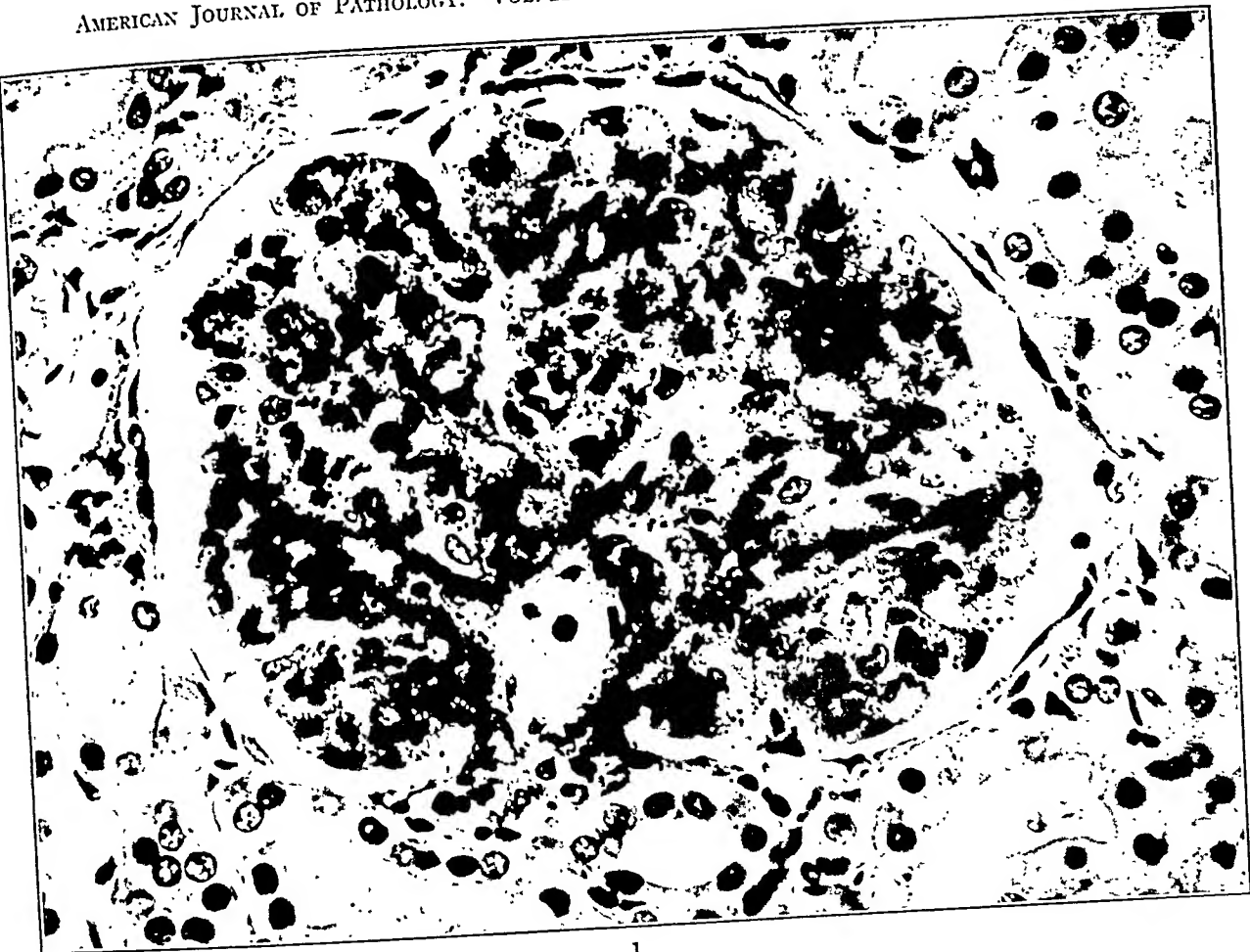
FIG. 2. Shows metal particles in the connective tissue under the epithelium of the renal collecting tubules in a pyramid. $\times 500$.

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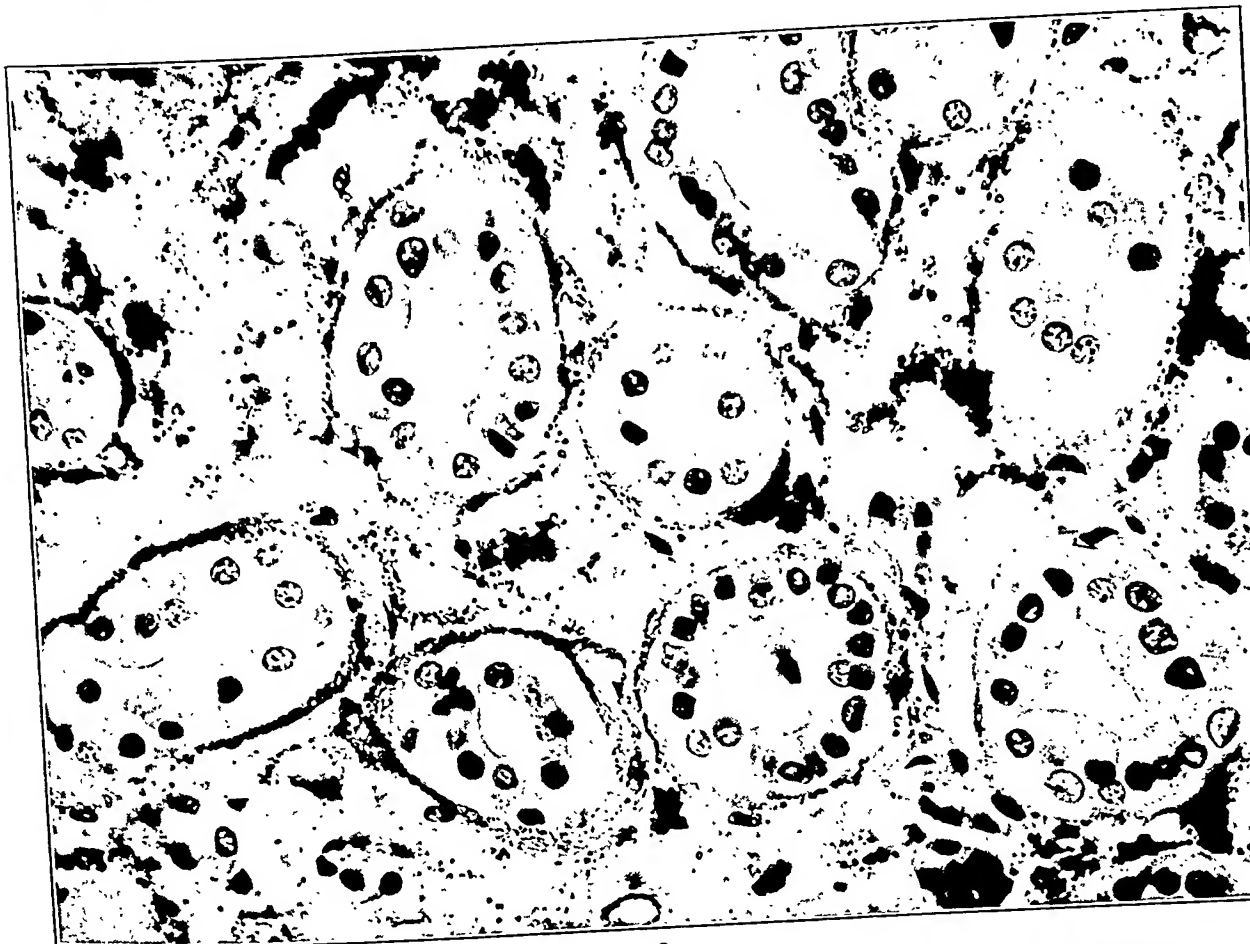
PLATE 167

FIG. 3. Silver granules scattered throughout the connective tissue of the liver capsule, following the distribution of the elastic fibrils. $\times 500$.

FIG. 4. Silver around a bile duct in the fibrous tissue of a portal area of the liver. $\times 500$.



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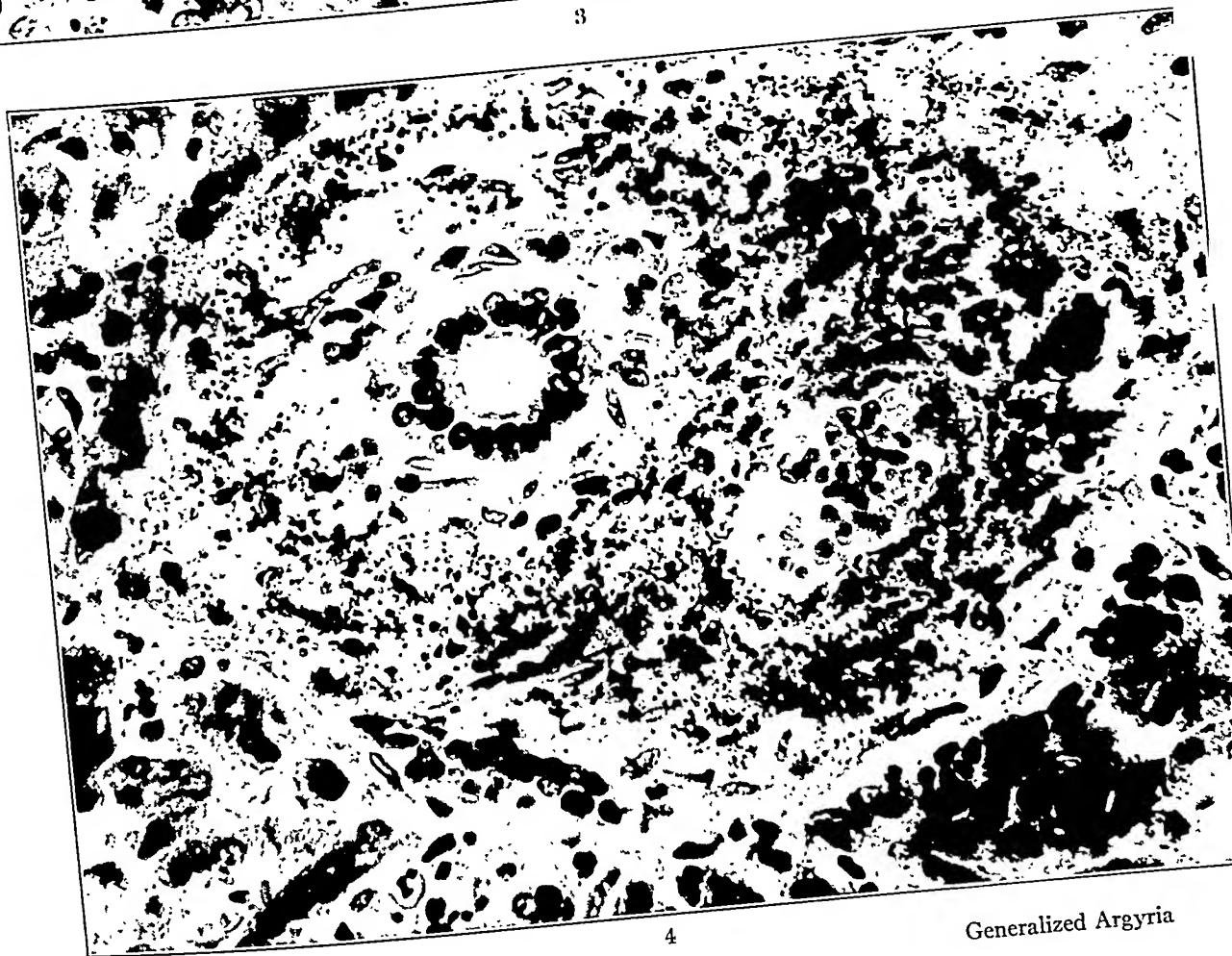
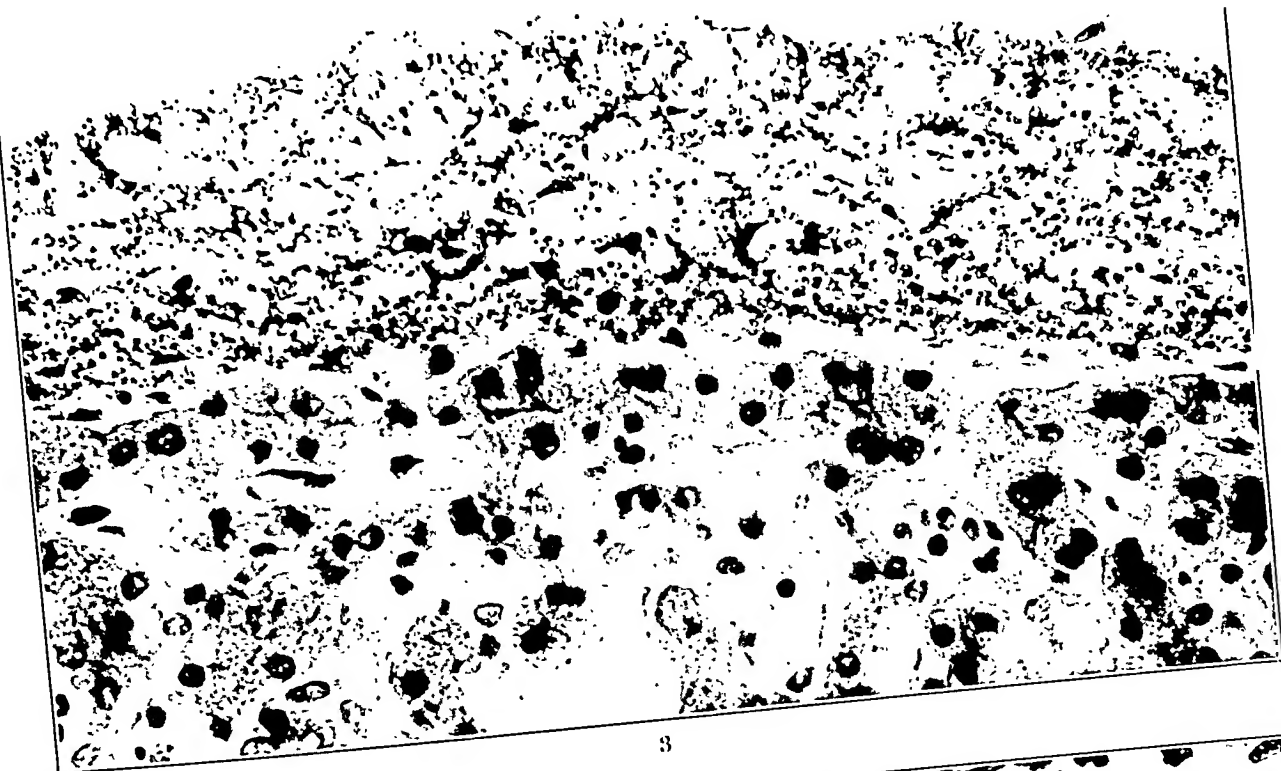


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PLATE 168

FIG. 5. Granules of silver in the periductular connective tissue of the pancreas. $\times 500$.

FIG. 6. Stomach showing metal granules distributed irregularly through the muscularis and massed in the connective tissue close to the epithelium of the glands. $\times 500$.



Generalized Argyria

PLATE 169

FIG. 7. Skin with silver particles in groups in the corium.

FIG. 8. Coil glands of the corium demonstrating metal in the connective tissue adjoining the epithelium. $\times 500$.

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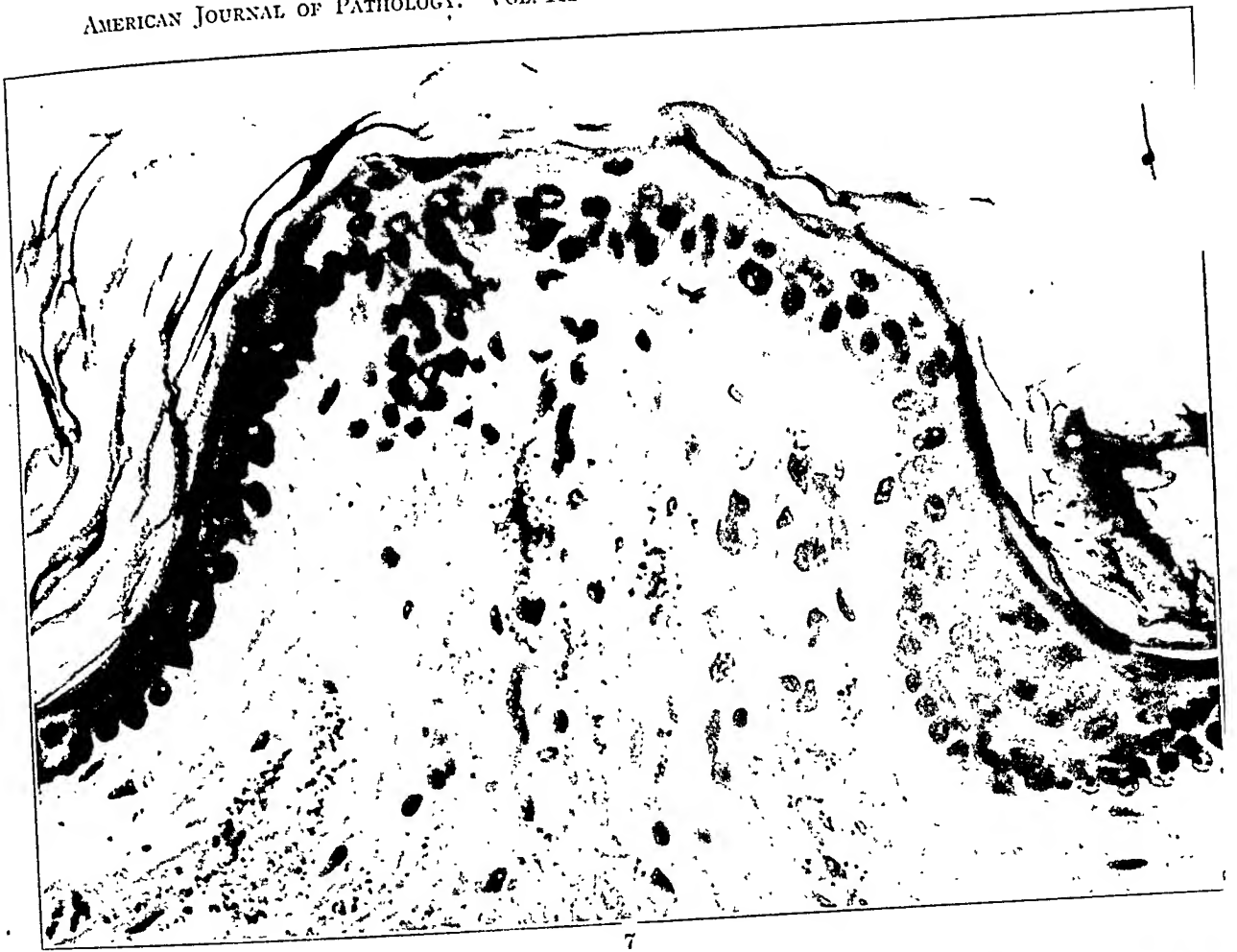
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Generalized Argyria

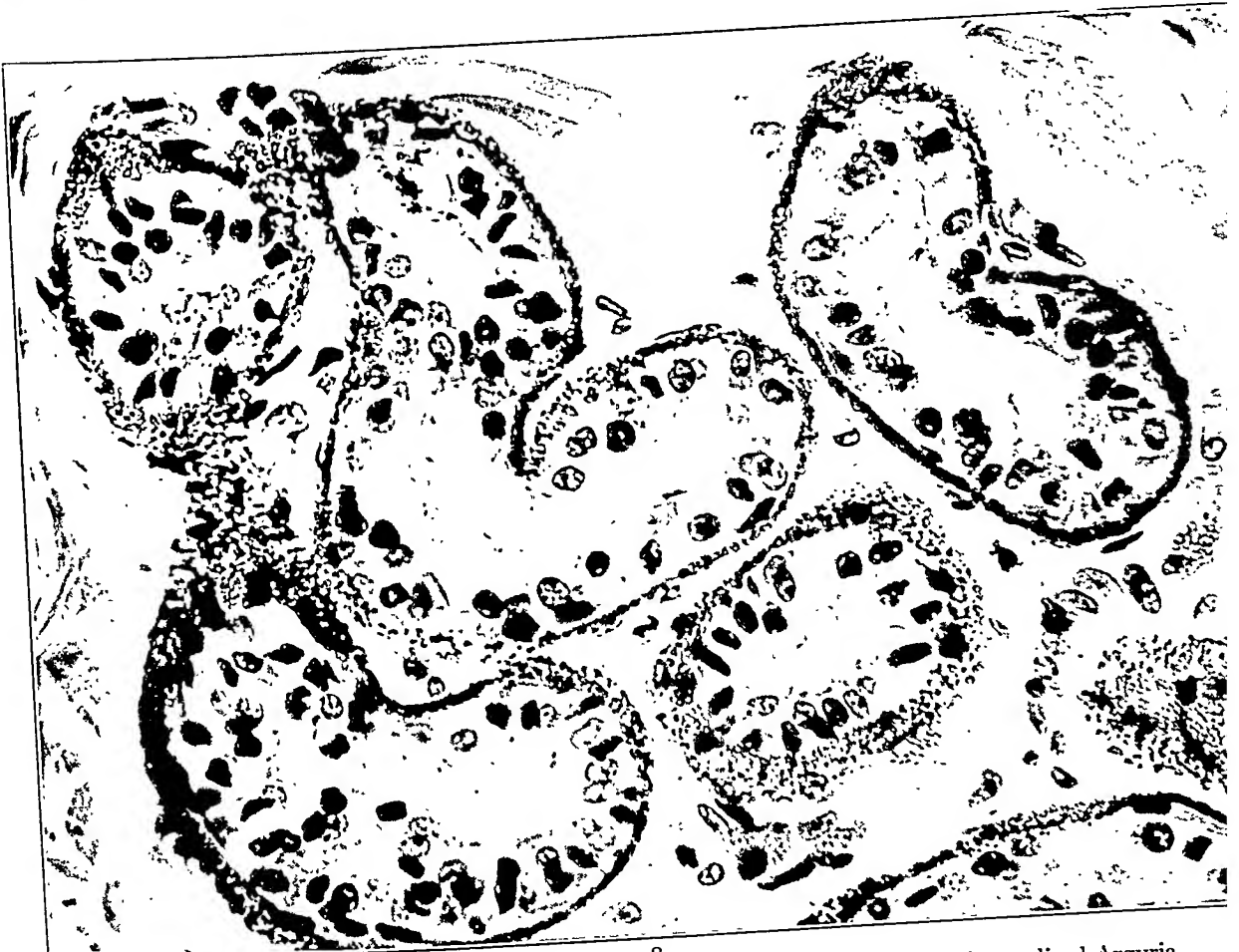
PLATE 170

FIG. 9. Heart muscle with silver granules in the fibrous tissue between the muscle fibers. $\times 500$.

FIG. 10. Lymph node showing much silver deposited around lymphatics outside the capsule and also within the pulp. $\times 250$.



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